

Prosperity and Science as Chicken and Egg

SIR ALEC CAIRNCROSS, President of the British Association at its annual meeting last week, has probably lost a good many friends among professional scientists with his stern declaration that postwar British governments may have been misguided in supposing that investment in scientific research would ensure prosperity and competitiveness with advanced economies such as that of the United States. After all, a great many people have invested their reputations in various versions of this belief, and nobody likes to be told that he has backed the wrong horse, especially when so much time has gone by that he personally may be unable to back another. It is also unfortunately true that much of what has been written in the past, and many government policies on research and development, have been based on the assumption that it might be possible to win extremely quick economic returns from investments in research and development, and to that extent Sir Alec's declaration, reactionary though it may have seemed to some, is entirely well grounded. The expectation that investment in research and development on nuclear power stations in the late fifties would create an internationally dominant nuclear power industry was one of the more conspicuous miscalculations. But it would be a great misfortune for everybody if Sir Alec Cairncross's complaints that people have expected too much too quickly of scientific research and development should confuse the issue of what connexion there may be in the long run between research and development on the one hand and prosperity and amenity on the other. In particular, it is to be hoped that Lord Rothschild and his helpers, now by all accounts engaged on a comprehensive review of the British government's involvement in research and development, will not use the spirit of Sir Alec's declaration as an excuse for parsimony.

Much of the growth of scientific research in Britain in the last few decades stems from the way in which, in the late forties, a succession of public inquiries drew attention to the great scarcity of skilled manpower in large parts of British industry. But this was also a period in which governments and others hoped that it might be possible in the postwar world to stimulate deliberately the kinds of developments which had given Britain a great reputation for scientific innovation during the Second World War—radar, jet engines and such things as penicillin. Sir Alec Cairncross pointed out last week that Whittle, the man who made the first jet engine, was not a scientist in the ordinary sense but, rather, a man in the more straightforward tradition of the classical inventors. Moreover, the argument goes, jet engines saw the light of day not merely because Whittle was an enthusiast but also because there was an urgent military need of such a development in technology. The implication, of course, is that consumer demand will somehow evoke appropriate technological developments from the community of inventively inclined people, and there is of course some truth in this description of the incentives for invention. How else is it possible to explain the way in which many new develop-

ments must be reinvented on half a dozen occasions before they find an economic climate in which full-scale exploitation becomes profitable? From this point of view, the British government's investment in research and development in nuclear power was frustrated not so much by technical difficulties as by the lack of a market. Yet none of this implies that the recognition of the need for some technical development will instantly insure that the necessary inventions are whistled up from thin air, as it were. On the contrary, there is a host of circumstances in which an apparent need for a new technical development has been satisfied not by the local invention of a suitable device in Britain but, rather, by its invention elsewhere. Questions about the relationship between investment in research and development and industrial innovation must be answered by a definition of the economic and social circumstances in which need will stimulate the appropriate developments. The question has been too much ignored.

The most obvious danger, and the most common fault, is to make too sharp the connexion between scientific research and the social or economic need that it must satisfy. This was the British government's fault with nuclear power. Now that advanced communities acknowledge that motor cars can clog up the streets of cities, there is a temptation to spend money on the development of hardware for other kinds of transport such as railways or travelling pavements. Similarly, because exhaust fumes are a nuisance, there is a temptation to spend money on steam engines and electric storage batteries. Yet it may well be that in the long run cities will benefit more from building techniques that make possible and cheap a more complete separation of people and transport, or even from systems analysis that makes transport in the sense of the physical movement of people from one place to another less necessary than at present. It is even possible that the best cities will in future owe less to research on transport as such than to the embodiment in working systems of some of the techniques of electronic communication which have already done much to replace messenger boys by switchboard girls and which still have a long way to go. This is eminently a field in which an attempt to meet a proper and clamant demand for cities that are better organized may encourage inappropriate innovations. Everything depends on whether the vision that inspires the research is of the right breadth, neither too pragmatic nor too abstract.

Strictly industrial developments, electronic computers for example, provoke or should provoke similar considerations. One of the minor scandals of the 1960s is that computer manufacturers made and sold all kinds of equipment with great promises of immediate economic benefit but without means of making sure that the customers would reap the benefits they had been promised. The trouble, in retrospect, is not simply excessive enthusiasm among salesmen but also a failure by computer designers to appreciate the complexity of many of



the tasks for which they had commended their machinery. On the face of things, of course, it is paradoxical that there should be few complaints about the way in which computing machinery functions in complicated military innovations such as anti-ballistic missile systems, and endless complaints at the way in which computers function when dealing with practical commercial problems such as the calculation of weekly payrolls, but this should be no surprise; the second situation differs from the first in that it requires computing machinery to be made to play a part in a framework previously designed entirely for people, and to do this for comparable cost. But the persistent failure of computer manufacturers to live up to their promises in the past few years is of course also a definition of a technological need, and there is no doubt of handsome profits for whichever manufacturer of computers finds himself able to make machinery that functions as it is said in the brochure to function.

In countries such as Britain, there are particular economic and social needs which cry out to be satisfied. What is to be done about shipbuilding? Does Britain, either separately or as a part of the European Community, have a future as a manufacturer of aircraft or of the electronic systems with which modern aircraft are filled? Is there some way of making the automobile industry competitive in circumstances in which it seems all too probable that manufacturers on the mainland have already won the race, both in technical quality and in the cost of production? And if sober consideration does suggest that traditional industries will have very little to contribute to British success in the European economic competition for the few decades during which the European Community will be a club of economic competitors, not a federation of like-minded states, what new industries will give the British economy grist for its mill in the years ahead? It is impossible to see how any of these questions could be answered without an intellectually strong scientific community in Britain able to provide the men and women capable of solving not merely the problems which exist but those which have not yet emerged.

Quite apart from over-optimism about the probable connexion between research and prosperity, the past few decades have seen in Britain the reflexion in science policy of some of the problems which have confused international relations as a whole—the tendency to believe that even small countries such as Britain could remain creative and successful in all possible industrial fields, the tendency to suppose that native wit and intelligence would be a match for the systematic deployment of imagination and skill, backed by money, in countries such as the United States, and the suspicion that the inherent virtues of British manufactures would somehow make unnecessary attention to such skills as marketing, still regarded in Britain as techniques appropriate to second-rate commodities. In the past few years, as luck will have it, British society seems to have acknowledged that these principles are now untenable. One of the dangers of too literal a reading of what Sir Alec Cairncross had to say last week is that the tendency towards braggadocio in British science policy, which he has mistaken for a more pedestrian if less reprehensible wish to turn research into gold, may now be replaced by the equivalent in research policy of what the Little Englanders would do in politics. In short, there is a danger, which the present government so far has given no sign of acknowledging, that public

policies on the scale and balance of research and development will in future be guided by the most pragmatic and expedient calculations.

This is why the British government, and Sir Alec Cairncross as well, should read carefully the speech on Monday this week in which Sir Brian Flowers, chairman of the Science Research Council, made the first official attempt so far to anticipate the effects on British policy for research and development of entry into the European Community. Over the years, British governments have taken an entirely misguided view of what the European relationship entails. A decade ago, when the first attempt was being made to win entry into the Common Market, people such as the then Minister of Aviation, Mr Peter (now Lord) Thorneycroft, hoped that it might be possible to make the process easier by offering the rocket Blue Streak as a basis for the development of the European Launcher Development Organization. By now, there is plenty of evidence that Mr Thorneycroft's calculation was mistaken. In Mr Harold Wilson's time, the British government offered an even more ambitious version of the same prospectus, proclaiming the advantages of what it called a technological community in Europe, but what was intended was simply a more grandiose system in which European countries would collaborate with each other on a multitude of projects, no doubt hoping against hope somehow to resolve the perennial problems of how best to share out contracts and kudos fairly among participants and non-participants.

The merit of what Sir Brian Flowers has to say is that it recognizes the importance (see *Nature*, **230**, 198; and **231**, 3; 1971) that European collaboration should allow participants to concentrate on the things that they do best without fearing that competitors will in the process steal a march on them. Although most discussions of British membership of the European Community have been based on the assumption that the six present members, and the ten that there will be if the new applicants eventually join, are a group of nations bound together by artificial rules in such a way as to make economic competition more wounding than it would otherwise be, the truth is that the European pattern is one that may enormously simplify the European economy. The fact that tariff barriers disappear implies that once chauvinism has been eroded by the passage of time, customers will buy the cheapest products without concern for where they come from, and industries will therefore find themselves concentrating on a few manufacturing plants, not a random scattering throughout Europe. But in this state of affairs, it will be important that there should be opportunities for skilled technical people to move freely to where their ingenuity can best be employed (which is also one of the conditions of the Treaty of Rome) by which time it will be apparent that if British industry is unable to use the skills of its graduates in science and technology, other countries will be eager to do so. But from this it follows, as night from day, that attempts will have to be made to coordinate the policies on research and development which the separate governments pursue. In the long run, the result is bound to be an integration of the university systems as well as of policies for research and development. The British government is lucky that Sir Brian Flowers has now pointed out the need for moving in this direction. It is unfortunate that Sir Alec Cairncross had nothing to say on the subject last week.

Where Real Trouble Lies

IN the past several months, all kinds of fears have been expressed about the damage that might be done by pollution of various kinds to species of animals which are either of economic importance or of some other value to society at large. By now, for example, it is known that DDT and other organochlorine pesticides can damage the reproductive capacity of birds, particularly falcons and eagles. Similarly, there have been fears that pesticides and other chemicals in the sea may cause irreparable damage to breeding stocks of fish or other sea animals. It is therefore ironical but important that signs are now rapidly accumulating to show that the most serious and rapidly growing threat to the survival of these species lies not in any form of pollution but in the activities, sometimes illegal, of people armed with nothing more unfamiliar than guns and trawling nets.

The case of the American eagle is already something of a scandal. Over the years, the populations of the bald eagle and the golden eagle have steadily declined to 2,000 and 8,000 respectively and, because both birds are protected by federal legislation, it has been assumed that the decline in their populations is to be attributed to the hostile influences of the contemporary environment, DDT and all. But now it turns out that farmers in states such as Wyoming, anxious to protect flocks of sheep and other livestock against predators, have been hiring helicopters so as to destroy eagles in flight. One estimate is that close on a thousand eagles have been destroyed by these techniques in Wyoming in the past year, and it is clear that others have been killed by poisoned bait of various kinds. On the face of things, at least, it looks very much as if the illegal operations of the farmers are in themselves sufficient to account for the decline in the populations of the eagles, and that they are sufficient to jeopardize the future of the two species. Because there are bound to be serious limits to the efficiency with which the law can be enforced, is there not a strong case for asking that some of the ecologists who worry about the effects of pesticides on the breeding stocks of eagles in North America should turn their attention to such a close understanding of the habits of these splendid creatures that means might be evolved for keeping them out of reach of their most serious enemies, the farmers of the north-west?

The case of vertebrates in the North Atlantic is potentially much more serious. It is already well established that at least two species of whale have been exterminated as a result of hunting in Antarctic waters, and others are in danger (see *Nature*, 232, 80; 1971). There is now a serious danger that species of commercial fish may be exposed to insupportable pressure from fishing vessels. It is already well documented that the catch of herring in the North Sea and around the British Isles has declined steadily since the Second World War, and this is one of the reasons why the British government is sensitive about the limits preserved for national fishing in coastal waters, due to be negotiated in Brussels this week with the members of the European Community. But it is also becoming harder for fishing vessels to keep up their productivity in catches of cod and haddock, two of the principal catches in the North Atlantic. Figures recently made available by the Fisheries Research Board of Canada (ref. Tech-

nical Report 260) illustrate how sharp has been the decline of the population on what is called the Scotia Bank, the shallow region of the continental shelf east and south of Nova Scotia. By means of comprehensive samples in the region, it has been possible to show that for both species, the present rates of exploitation are comparable with or even exceed the maximum sustainable yield of the two species. But there is at present no limit to the intensity of fishing in regions such as this; instead, there is a constant incentive to travel further afield in search of fish. Although not nearly enough is known of the population dynamics of cod and haddock, there is at least a strong danger that over-fishing could bring about a marked reduction of the stocks of fish available within a decade.

One obvious moral is that some attempt should be made to set up international controls of the intensity of fishing in the North Atlantic. If the snail's pace at which similar attempts to restrict the sovereignty of nations and the independence of fishermen is any guide, a decade leaves hardly any time for manoeuvre. The second important lesson is that those who fear the way in which pollution of various kinds may damage marine life should acknowledge that whatever the rightness of their case, there are still more urgent problems to be tackled.

100 Years Ago



The Aurora

I HAVE just read Mr. Wilson's interesting paper entitled "Some Speculations on the Auroras," published in your periodical for September 7. In the *Philosophical Magazine* for July 1870 I made a suggestion as to the origin of auroras similar to that just published by Mr. Wilson.

The periodicity in auroral displays noticed by Mr. Wilson had not attracted my attention. It would doubtless, if it were well established, be confirmatory of the views independently put forward by Mr. Wilson and myself.

A. S. DAVIS

Meteor

ON Saturday, September 2, at 8.14 or 8.15 P.M., I saw a fine meteor under very favourable circumstances. I was standing with several friends at the door of Mr. W. F. Moore's house at Croakbourne, in the Isle of Man, and we were looking up at the western sky at the moment when the meteor came. It started between, I think, γ and π Herculis (it was too cloudy to see those stars), descended nearly vertically, passing through Corona Borealis, and vanished a little below ζ Bootis, at about 15° above the horizon. It moved slowly but continuously, taking from two to three seconds in travelling over 45° . It broke into three, which followed one another, connected and followed by a luminous train which was visible for about one second. The first part of the three was brilliant white, and was estimated by Mr. A. W. Moore and myself independently as equal in size to $\frac{1}{4}$ th of the moon's surface. It was very brilliant, being mistaken by the Rev. John Howard, who was looking in another direction, for a flash of lightning. The two latter globes were blue.

Rugby, September 6

J. M. WILSON

From *Nature*, 4, 385, September 14, 1871

OLD WORLD

BRITISH ASSOCIATION

Cross Roads Again

from our Special Correspondent

Swansea, September 8

THE 135th annual meeting of the British Association, now finished, has been more than usually a demonstration of how much the association has to offer and a source of anxiety that it might not be able to continue. The scientific programme has been imaginative if unspectacular. The association has also gone a long way to meet the criticism that it has done too little in the past to provide a forum for the discussion of important public issues in the management and exploitation of science. But a cloud, now very much bigger than a man's hand, continually hangs over the association's future. It remains to be seen whether the decisions taken this week will provide an administrative framework in which the association will be able to survive.

Letter from Rothschild

SIR,—In your leading article on August 20 you referred to "inexcusable delays" on the part of the Government in reaching important decisions. You cited the study of certain aspects of Government research and development.

The Central Policy Review Staff was asked by the Government at the end of March to report on this subject by October. Given the complexity of the issues involved, to which your article testifies, six months is surely not too long for a study of the organization and management required for the annual expenditure of £650 million of public funds. This study has obviously entailed "the ample discussion with interested parties" which you imply the Government has been neglecting.

You also argue that in the management of the Government's policy on research and development, "the machinery that exists is a hindrance, not a help". It is precisely this question of the machinery which the CPRS has been studying.

Yours faithfully,

LORD ROTHSCHILD

11 Herschel Road,
Cambridge

It is good to know that the Rothschild inquiry is well on with its work, but must everything wait until its report has been digested? And will the Dainton report on the Research Councils be published?

EDITOR.

The mood of the annual meeting, a cautious blend of enthusiasm and scepticism, was in part determined by the opening address of the president, Sir Alec Cairncross, who argued that economic history contains very little evidence to suggest that "the process of innovation owed much directly, even if it owed a great deal indirectly, to advances in scientific knowledge". The result, he said, is that those "who put their faith in science as a means of our economic salvation exaggerate the part played by the body of formulated knowledge in the generation of technological change". He went on to say that it is consumer demand—the interests of governments as well as of individuals—and not advances in scientific knowledge that "gives innovation its motive-force", and that in the moulding of innovation by consumer demand, what matters most is the way in which industrial management exploits the potentiality of techniques.

The moral which Sir Alec Cairncross drew from this analysis is that there is no correlation between expenditure on research and development and the rate of economic growth. The suspicion that there must be has, in Britain, moulded government policy towards innovation in such a way that "the problem was misconceived, the strategy mistaken, the tactics unsatisfactory . . .". For the British and for other governments, the social climate is also an important determinant of the pace of innovation. Britain has been unlucky in this respect. But for all governments, according to Sir Alec, innovation "comes far more from below than above". Governments may be able to cajole but they cannot direct.

Sir Alec's declaration has drawn a number of opponents in the past week. At a public discussion on Monday evening, Sir Brian Flowers, the chairman of the Science Research Council, said that he could have written a similar address to the Royal Institute of Economics, replacing the word "science" by "economics". Sir Alec himself is anxious to insist on the distinction between science and technology, so that in that sense his argument is not relevant to all the things that British governments have done in the past few years to foster innovation in industry.

One of the ingredients missing from Sir Alec's address was a sense of what might happen or should happen in the planning of government policy. From a quite different point of view Sir Brian Flowers spelled out on Monday some of the steps which in his view must be taken to foster the development of science within the European Community, with luck enlarged by the accession of Britain and the other applicants. (The full text of this address will appear in *Nature* in the near future.) On the creation of common institutions

he asked that innovators should insist "the science must be good science" and the purpose of the institution must be clear. But the initiative for creating a new organization must come from scientists and there must be good reasons for thinking that international collaboration is essential. With these conditions satisfied, Sir Brian considers that there are great benefits to be won in the development of the European economy, especially if ways can be found of encouraging the nations of Europe to "give up the idea of alternative and purely national ventures".

Elsewhere, various sections of the association have sought to lay down the law on what should be done to brighten or to make more liberal the education of science graduates at British universities—these days, it seems, the advocates of liberal courses are the only ones who dare to speak in public. Several of the meetings in the past week have been designed to draw attention to social problems inherent in scientific developments of all kinds, and there has been a particularly successful discussion, led by Professor R. R. Porter, of the social problems occasioned by medical research. The same question was argued over at a discussion to which members of the general public were invited—an innovation for the British Association.

Controversy about the place of science in the modern world has sprung up at several sessions, sometimes by accident and sometimes by design. Nobody is in a position to count heads and thus identify the winner. On balance, however, this year's meeting has been distinguished by much more open discussion of the social antecedents of science, which is why the statement issued by the British Society for Social Responsibility in Science that "a few tame symposia on environmental things, a few public lectures on the ethical problem of this or that are not a real response to this crisis" has fallen a little flat.

This "crisis" is whether the British Association will somehow manage to survive. The association seems to have gained very little from the year long study by the Committee of Review (see *Nature*, 231, 412; 1971). The financial position is now even more desperate.

RADIO TELESCOPES

Jodrell Bank in Wales

by our Astronomy Correspondent

THE Science Research Council has earmarked £250,000 for a detailed design study of the telescope which the Nuffield Radio Astronomy Laboratory at Jodrell Bank, and in particular the laboratory's director, Sir Bernard Lovell, have looked forward to building for years. But it is clear that Sir Bernard's original aspirations have had to be toned down some-

what—instead of the 400-foot dish the Jodrell Bank group had hoped to build, the design study is for a telescope with an aperture of 375 feet. Steel prices have also made it economic to replace much of the steel in the original design by concrete.

Nevertheless, the Mark VA, as it will be called to distinguish it from the original design, will be the largest fully steerable radio telescope. Its diameter will be 47 feet greater than the current holder of the record, the telescope that is at present being brought into operation at Effelsberg, West Germany, by the University of Bonn.

But the design philosophy of the new Jodrell Bank telescope will be radically different from that of the Bonn telescope. The Bonn telescope has a light structure which is designed to distort under the forces of gravity and wind loading in such a way that the surface always remains a paraboloid to a sufficient accuracy for observations down to wavelengths of a few centimetres. In the design for the Mark VA the effects of wind are to be reduced by using a heavier structure, and the position of the reflecting membrane will be adjusted during operation by means of screw jacks attached to the backing structure to allow for gravity distortions at different elevations.

The steel backing structure which will carry the membrane of the Mark VA will pivot in elevation on bearings supported by a beam which in turn will be supported by a concrete turntable. It is hoped that the telescope will be operable down to three centimetres.

Last week's announcement by the Science Research Council should put European radio astronomers at a further advantage compared with radio astronomers in the United States, where the lack of new radio telescope projects is widely lamented. In Europe, on the other hand, significant new telescopes have recently begun operation at Westerbork in Holland and at Effelsberg, and a three-mile interferometer is under construction for Sir Martin Ryle's group at Cambridge. But it will clearly be past the middle of the decade before the Mark VA could be completed—it will be a year before the design study will have progressed far enough for tenders to be invited, and it is expected that the telescope will take three or four years to build, at a cost that is likely to be more than £4 million. Consulting engineers for the Mark VA will once again be Husband and Company, who were responsible for the 250-foot telescope at Jodrell Bank.

Although most radio astronomers will undoubtedly welcome last week's news, some astronomers have argued that the wealth of instruments becoming available to radio astronomers in Europe makes the construction of a radio tele-

scope able to operate down to millimetre wavelengths more urgent than the Mark VA (see *Nature*, **227**, 107; 1970). Such a telescope would primarily be valuable for work on interstellar molecules which have characteristic lines at centimetre and millimetre wavelengths, and would allow European astronomers to participate in a field which up to now has been dominated by the Americans. It is understood, however, that such a telescope may be under consideration by the Science Research Council, although for reasons of atmospheric transparency it could not be situated in Britain.

The site chosen for the Mark VA is at the village of Meifod in Montgomeryshire, fifty miles from Jodrell Bank, and to a considerable extent the telescope will be controlled automatically from Jodrell Bank. Land was bought at Meifod three years ago for the laboratory, with a view to Meifod being the site of the Mark V telescope, although planning permission for the dish has not yet been applied for. Although the people of Meifod are reported as being in favour of the plan, Meifod also happens to be the headquarters of the Council for the Protection of Rural Wales, which has made available the following statement: "The telescope will be completely out of scale with the Meifod valley, a countryside of small and intimate detail and exceptional beauty. The nearest hill to the site rises little more than the diameter of the dish.

"Sir Bernard has said recently that the whole of Britain was searched for a suitable site. We understand that only scientific criteria were taken into consideration in these searches and the appearance of the landscape was ignored. No consultations were held with the Countryside Commission. Local opinion is in favour of the telescope but it is now realized that our countryside is a matter of concern for everyone in Britain. Local opinion believes that Meifod will be 'put on the map'. However, the benefits may be illusory since employment will be minimal once the site is built. Sir Bernard has told us that tourism will not be encouraged because of electrical interference and tourist facilities have already been provided at Jodrell Bank. The danger of electrical interference will also inhibit the natural development of the area within range of the site."

POLLUTION

Canberra Conference

from a Correspondent

SOME of the guidelines for the long awaited United Nations Conference on the Human Environment to be held in June 1972 in Stockholm became clearer

during a thirteen nation meeting of the UNESCO advisory committee held in Canberra on August 23–27. The recommendations of the committee on Natural Resources were couched in general terms but they give some idea of the directions which UNESCO would like the Stockholm conference to take.

The committee proposed that interdisciplinary projects should be emphasized including both social and natural sciences and that priority should also be given to continuing and expanding those projects begun under the aegis of the International Biological Programme which have proved to be successful. The committee also favoured an early start to some projects which are likely to be productive in order to get the forthcoming "Man and the Biosphere" programme, that is being sponsored by UNESCO, firmly established. Also it recommended that projects should "concentrate on the study of dynamic rather than static phenomena".

The committee further recommended that a set of ecological guidelines for development projects should be prepared to encourage planners of major works, such as large dams, to include ecological considerations in their planning. The director of UNESCO's Natural Resources Division, Dr Michel Batisse, said that he hoped that UNESCO would publish some definitive guidelines after the Stockholm conference. The administration of Man and the Biosphere was criticized by the advisory committee, as was the administration of the International Geological Correlation Programme (IGCP). The committee recommended that the IGCP administration should come under a single coordinating body whose members would be appointed jointly by the Director-General of UNESCO and the President of the International Union of Geological Sciences (IUGS). The IGCP is a joint project of UNESCO and IUGS that is planned to begin in 1973. Its aims are to correlate geological knowledge of different areas—partly in the hope of encouraging new mineral finds. These recommendations will be put to an inter-governmental conference to continue preparations for the IGCP which will be held in Paris on October 19–29.

Dr Michel Batisse said that Man and the Biosphere was receiving strong support from developing countries, communist countries and most western nations but that the United States appeared only moderately enthusiastic with Britain also treating the programme very coolly. Professor J. N. Black of the Forestry and Natural Resources Department, Edinburgh University, was elected chairman of the advisory committee, which possibly signifies an increase in Britain's support.

SRC Members

THE Secretary of State for Education and Science, Mrs Margaret Thatcher, has this week appointed two new members to the Science Research Council to replace Professor Sir Ronald Nyholm and Professor P. A. Sheppard, who have retired from the council. They are Professor H. Elliot of Imperial College, London University, and Professor R. Mason of the University of Sussex. The composition of the council from October 1 will then be: Professor Sir Brian Flowers (chairman), Dr A. H. Chilver, Dr D. S. Davies, Dr E. Eastwood, Professor H. Elliot, Professor H. Ford, Professor J. C. Gunn, Professor F. Hoyle, Professor H. L. Kronberg, Professor R. Mason, Professor P. T. Matthews, Dr J. W. Menter, Professor E. W. J. Mitchell, Mr D. L. Nicolson, Dr E. J. Richards and Professor M. M. Swann.

NUCLEAR REACTORS

All Set at Dounreay

ALTHOUGH the construction of the British prototype fast breeder reactor is running about eighteen months behind schedule, it is still ahead of similar projects in other countries. The prototype is being built by the Nuclear Power Group at the United Kingdom Atomic Energy Authority Experimental Reactor Establishment at Dounreay in the North of Scotland, and should be completed at the end of this year.

The original plan was that the reactor system should deliver power to the

North of Scotland Hydroelectric Board during the first half of this year, but because of trouble with the construction of the roof of the reactor vessel, commissioning is now expected to take place early in 1972 with the first output of electricity at the end of the year. By contrast with the smaller Dounreay fast reactor which first went critical twelve years ago and has been producing about 14 megawatts (MW) of electricity as well as providing a valuable irradiation facility, the prototype fast reactor (which has cost about £38 million) will generate at least 250 MW and earn about £4 million a year from electricity sales. A further £2 million a year will probably accrue from rental of irradiation space in the core.

The Dounreay prototype fast reactor represents an important link between the small experimental fast breeder and the reactors needed for a new generation of nuclear power stations. The Central Electricity Generating Board is seriously considering the feasibility of fast breeder reactor stations and proved commercial systems could also be sold abroad provided that the designs are not so geared to the requirements of the Central Electricity Generating Board that they are unattractive to other potential customers (a situation that may account in part for the poor response to the British advanced gas-cooled reactors). Discussions between the Atomic Energy Authority and the Central Electricity Generating Board have led to a proposal that a 1,300 MW fast reactor power station should be started in 1974. This would cost about £130 million, but any decision to go ahead with such a project cannot be taken until the satisfactory operation of the Dounreay prototype fast reactor is assured and the thorny problem of

siting fast reactors near centres of population is at least partially resolved. The Central Electricity Generating Board would certainly not be happy with a remote station because of the high costs of electricity transmission. A definite choice of site for the first commercial fast reactor will, however, have to be made during 1972 if the rather ambitious 1974 deadline is to be met.

In spite of the interruptions in the Dounreay prototype reactor programme, only the Russian 350 MW prototype reactor at Shevchenko is being built on a comparable timescale; a full-size Russian system is, however, unlikely to be a serious commercial competitor in the West. A French 250 MW prototype reactor project is under way at Marcoule, but the core is not expected to go critical before 1973. In the United States General Electric and Westinghouse have designed a "demonstration" fast breeder reactor but construction has not yet started.

Britain is also involved in two collaborative fast reactor ventures in Europe. In 1969, the Nuclear Power Group joined representatives of eight European countries in a project to investigate gas cooled fast reactors (as opposed to the sodium cooled fast reactors such as the Dounreay prototype); the group is also involved with Kraftwerk Union (through its associated company Interatom), Belgonucleaire and Neratoom in an agreement to market fast reactors eventually on a worldwide basis. Interatom, Belgonucleaire and Neratoom are at present working on the design of a 300 MW fast reactor to be built in West Germany.

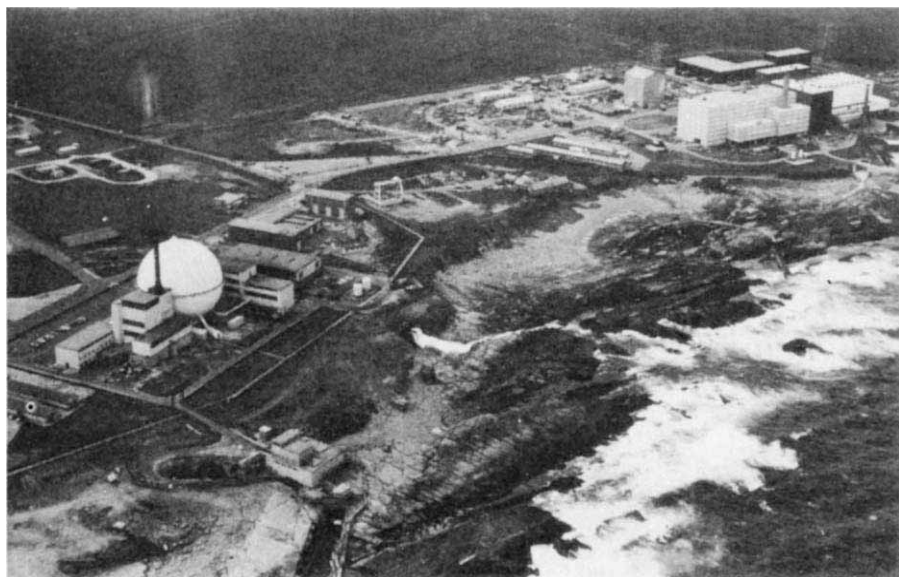
SPACE SCIENCE

Probing Mars

from our Soviet Correspondent

THE Soviet Mars-2 and Mars-3 probes, launched on May 19 and 28, 1971, and now half-way through their six-month voyage to their target, not only carry experiments for the study of Mars and circum-Martian space, they also have on board a number of experiments intended to operate during the voyage itself. These are intended to measure magnetic field strength, the flux of charged particles and study the solar wind.

The solar wind experiment, which appears to be the most important to the Soviet space programme at this stage, is designed to determine the velocity, temperature and composition of the principal components of the solar wind and the variation of these parameters with time and distance. It includes an ion-electron spectrometer unit, capable of measuring the spectral



The Dounreay Experimental Reactor Establishment. The prototype fast reactor is in the right background, and the older Dounreay fast reactor is in the left foreground.

distribution of the particles from 30 eV to 10 keV. This unit consists of eight individual spectrometers, each consisting of an electrostatic analyser, a high-gain electron channel multiplier (the first to be produced in the Soviet Union), sampling schemes and a signal recording device.

The first results from the solar wind experiment are now being analysed, and are providing good evidence of the satisfactory design of the unit. Some typical data so far obtained, for the first 10 days of the flight of Mars-3, are that velocity of the solar wind fluctuated between 300 and 600 km s⁻¹, relative content of alpha-particles varied from 5 to 10 per cent. The temperature of the solar wind particles and the considerable velocity gradients recorded are the subject of further and more detailed study.

The time-gap between the launching of the two probes and the availability of readings from other Soviet projects makes it possible to estimate which fluctuations are due to variation along the trajectory and which are due to variation in time. The solar wind data obtained from Lunokhod-1 and from the Earth-orbiting Kosmos satellites are providing reference data against which the Mars-2 and 3 data may be more clearly evaluated.

ENVIRONMENT

A-level Development

THE third attempt in recent years to develop an A-level syllabus in environmental studies was launched last week by a party of school teachers from Hertfordshire (*Environmental Studies—The Construction of an A-Level Syllabus*; National Foundation for Educational Research in England and Wales). The other two attempts, in 1968–69 and 1970, were unsuccessful but the present proposal has been constructed in greater depth. The Hertfordshire County Adviser for Environmental Studies, Mr S. McB. Carson, said this week that he was optimistic that the present submission would be accepted by the examination boards and the Schools Council.

There are twenty-two schools and two colleges in Hertfordshire that are planning to start teaching the new syllabus in September 1972 if approval is granted by then, and there are four examination boards that have already been approached with a view to instituting the syllabus. These are the University Entrance and Schools Examination Council of London University, the Associated Examining Board, the Cambridge University Local Examination Syndicate and the Oxford Delegacy of Local Examinations. With the exception of the Associated Board which is

not sympathetic to the new syllabus, the other boards are considering the proposal. Before the syllabus can be instituted, however, the boards must submit it to the Schools Council for approval. If approval is granted it is unlikely that more than one examination board will eventually offer the examination and arrangements will probably be made, as is currently done in other subjects for that one board to offer the examination to the schools attached to the seven other boards.

The development of an A-level course in environmental studies is a natural consequence of the O-level and CSE examinations in similar subjects that are already recognized by several examination boards. A problem in instituting the syllabus at A-level will be to find qualified teachers. Mr Carson said this week that the plan in Hertfordshire is to have the teaching done by biologists and geographers, or by specialist environment teachers—either those who are now involved in teaching the O-level subjects or by graduates of one of the several universities that now offer courses in environmental studies. The overlap of the new syllabus with other topics already being taught in schools is not a problem, according to Mr Carson, and of the fifteen university faculties that have in principle approved of the subject as an entry requirement, only one expressed reservations about a candidate offering both environmental studies and biology.

One of the arguments in favour of the new syllabus, says Mr Carson, is the increasing awareness of today's sixth-formers about ecological problems. Unlike most A-level syllabuses, A-level environmental studies will be as relevant as anybody could wish. Another potential advantage of the syllabus is that it offers career opportunities at non-degree level in teacher training and in Town and Country Planning.

BIOMEDICAL RESEARCH

Science Looks Forward ?

by our Special Correspondent

"Too plainly we are—in common with the whole of humanity—confronted with the question of where (the development of biology and medicine) leads." This was the reasoning which led Hoffman-La-Roche to celebrate their 75th anniversary by holding a symposium, "The Challenge of Life", in Basle last week. The aim of the symposium was outlined by Dr Alfred Pletscher (Hoffman-La-Roche) as "to define more clearly the problems created by biomedical progress and to find, if possible, essential facts or clues for their solution". Although the illustrious assembly of speakers, which included not only biologists but also sociologists,

philosophers and even theologians, was, of course, unable to provide definitive answers to this ambitious question, this proved to be one of the very few conferences where a consensus of opinion emerged to pinpoint with some clarity the essential problems which need to be resolved.

Some fascinating clashes of temperament dominated the symposium, in particular the conflict between the idealism of Dr Philip Handler (President, US National Academy of Sciences) and the pragmatism of Solly Lord Zuckerman (former Chief Scientific Advisor to the British Government). Whilst Professor Joshua Lederberg (Stanford University) and Professor Arnold Burgeon (Director, National Institute for Medical Research, Mill Hill) strongly urged that it is essential for scientists to communicate with the public and to reverse the alienation which many laymen now feel from science, Professor Niels Jerne (Director, Basle Institute for Immunology) maintained that it is impossible and, indeed, perhaps undesirable, for scientists to popularize their work.

Any curtailment of basic research would be tantamount to the book burning of the Nazis, said Dr Handler and Professor René König (University of Cologne), whilst others, in particular Professor Bernard Barber (Columbia University), were adamant that, to some extent at least, the sociologist, as a representative of public opinion, should be allowed to sit in judgment on the scientist. And Professor Margaret Mead (American Museum of Natural History) wished that the scientist would listen to the warnings that the social sciences can provide of reactions such as the backlash to science.

The consensus of opinion was with Professor Salvador Luria (Massachusetts Institute of Technology) that the critical point for control is not the direction of basic research but its technological development. And as Lord Todd (University of Cambridge) said, the problem of overpopulation loomed large over the entire proceedings. The right of the individual to expect medical science to maintain him in good health was defended by doctors, including Professor Burgeon and Max Lord Rosenheim (President, Royal College of Physicians of London), but the meaning of this was challenged by Professor Jeanne Hersch (University of Geneva). One of the main talking points was Lord Zuckerman's insistence that governments simply lack the resources to keep up with the demands for expensive treatments, and that medical science is aggravating this problem by devising more of these treatments.

A more detailed account of these discussions will appear in *Nature* next week.

GRADUATE EMPLOYMENT

A Question of Degree

CONCERN over graduate employment prospects and over the efficiency and effectiveness of the service that appointments boards provide marked the meeting of the Standing Conference of University Appointments Services (SCUAS) held in Cambridge last week. The conference, which meets every two years, was attended by 150 appointments officers and 70 guests, and took as its theme "Appointments Services in a Changing World".

Professor R. V. Jones, Professor of Natural Philosophy at the University of Aberdeen, outlined some of these changes in his opening address to the conference. After tracing the history of university education, Professor Jones suggested that the increase in the number of graduates had not been matched by an increase in quality. He went on to say that this factor combined with the present overproduction of specialist scientists will result in increasing numbers of science graduates following non-specialist employment.

To adapt to this situation science courses may have to be altered to produce more generally employable graduates. Professor Jones argued for a common course in the early stages of university education to prepare three types of graduates—those who would go on to be professional scientists, including teachers, those who would be professionally employed but not in the fields of research or design, and those who would almost immediately move outside science. He also suggested that demand would again equate to supply in the mid-1980s.

But, for the present, opinions varied as to the seriousness of the situation. Mr H. B. Putt, the retiring chairman of SCUAS, thought it possible that by the end of the year the situation "will be less disturbing than we had at one time feared". Mr Bernard Holloway, of the Manchester University Board, SCUAS's very own Job's comforter, felt otherwise. He predicted that by the end of the year the situation would be "worse than our worst fears", that 1972 would be at least as bad, and that 1971 was "the first of the seven lean years".

The duty of appointments officers, he said, was to be pessimistic, so that everyone became aware of the problems facing graduates. Mr Tom Snow, of the Oxford Board, while admitting that there are more graduates than traditional graduate jobs, denied that the situation was doomsday, and argued that "the function of education is not to fill gaps in the economic system". The impression must not be produced that higher education is a waste of money. Graduates, he felt, were still a very saleable product. He pointed out that by

1981, 30 per cent of men and 25 per cent of all eighteen-year-olds will be receiving higher education, and that the appointments boards should draw attention to the need they are, and will be, filling.

But it became clear from the conference that, saleable or not, to accommodate the growth in graduate numbers in the next ten years there will have to be a number of basic changes in attitude by students, schools, employers, unions and academics. All will have to become more flexible. University education will have, in the words of Mr Neil Scott of Nottingham University Board, to become an "education for citizenship", and much less a passport for a job. Graduates, particularly those who are not first-class material in either research or industrial terms, will have to be prepared to start at the bottom of their chosen ladder, and spend some years training. Students will have to realize this before they go to university, or, as Mr Holloway pointed out, graduates with unfulfilled expectations may produce a lot of resentment in society.

The expectation of what university has to offer will have to change at school level. This means alerting headmasters, of whom there were only two at the conference, as well as career teachers to the new realities. Academics too may have to be more flexible and provide broader based courses, and the conference will suggest to appropriate bodies that when new courses are planned appointments boards might have something to say. Employers will also have to accept that many jobs now filled by A-level entrants will in future be filled by graduates. Some employers are well aware of this—the GPO's traditional A-level white collar entry is now two-thirds filled by graduates.



Mr W. P. Kirkman, President of SCUAS, and Secretary of Cambridge University Appointments Board. "SCUAS", he says, "must be very well aware of changes, and move to meet them, but beware of rushing up any side track that comes along".

In fields where graduates have rarely if ever penetrated before there will have to be radical changes. Pay and promotion structures will have to be altered to allow graduate entry—at present their degree can put graduates automatically on to too high a pay and promotion scale for the employer to be able to afford to train them. Potential problems with unions who will object to the intrusion of graduates in their ranks will have to be ironed out.

But if the conference was aware of the limitations of other organizations, it was also aware of its own. The need for a central service unit which would provide badly needed centralized organization and prevent pointless duplication of work was reiterated, and it is likely that some such organization will soon be established. The American placement offices have had a central organization for the past fifteen years.

The use of a computer matching service, to cut out much of the routine paperwork, is being considered. This would allow the student to find early in the year what jobs are available in the fields in which he is interested. The system requires comparatively little personal information, and the National Union of Students, subject to certain safeguards, is cautiously enthusiastic.

A computer would allow more time for actual counselling, to help find graduates the kind of job suited to their talents, and, as Miss Audrey Newsome of Keele University pointed out, it is just that that the appointments boards must provide if they are not to become irrelevant to student needs. Miss Newsome also pointed out that appointments officers, although they are often experienced, are essentially an amateur body, and she called for training courses in counselling. Students, she said, must be met and helped before they come to feel that appointments boards are irrelevant.

Overall the conference was active without being pushed into panic by one particularly bad year, and they established a number of long-term studies to continue to examine their role. Their main problem is that while they have a close and constructive relationship with employers, particularly through the 300 members of the Standing Conference of Employers of Graduates, and, on the whole a good relationship with students, the appointments boards relationship with the universities, although improving is by no means uniformly good. They feel that now universities might listen more to what they have to say, and possibly spend more on them. The country spends about £80 getting a student to university, £3,500 educating him, and about £22 finding him a job. That balance is going to have to change if appointments boards are to provide the graduate with the service he needs.

NEW WORLD

AEC Causes Anger and Delight

by our Washington Correspondent

ACCUSED by the Court of Appeals of foot-dragging in its implementation of the National Environmental Policy Act, and of making a mockery of the act's intentions, the Atomic Energy Commission has reacted with a swiftness and boldness that suggest that the court hit a particularly sensitive spot. Little more than a month after being told by the court to take another look at its licensing policies, the AEC has come up with a new set of regulations which include every environmental safeguard that the court demanded, and which threaten to shut down five plants that are already operating and to halt construction on forty-six others while their environmental effects are reviewed. The new regulations also threaten to land the AEC in a flood of court actions brought by angry electricity companies who will have to foot the bill for costly delays and who face the prospect of adding expensive modifications to their plants to reduce environmental damage. When James R. Schlesinger, who took over as chairman of the Atomic Energy Commission on August 16, promised to be sympathetic to the demands of environmentalists, few expected him to demonstrate his sympathies quite so quickly or so dramatically.

The new regulations were made in response to a ruling of the US Court of Appeals which, on July 23, took the AEC to task for not considering environmental issues as a matter of course when an application to construct or operate a nuclear plant is given a public hearing; for excluding environmental issues from being considered in any public hearing announced before March 4, 1971 (fourteen months after the National Environmental Policy Act came into force); for failing to evaluate and balance certain environmental factors by abdicating its responsibilities to other agencies, and for allowing electricity companies to carry on construction of plants which have not been reviewed under the procedure laid down by the National Environmental Policy Act (NEPA).

It took the AEC more than eleven months after NEPA came into force to draw up regulations to comply with the act's intentions, but only one month to design new regulations to comply with the court's ruling. Some indication of the haste with which the AEC moved to meet the objections of its environmentalist critics can be gauged by the fact that when the new regulations were announced last week, the commission was unable to say how many plants at

present being constructed, or which have recently been given operating licences, would be affected by each section of the new regulations. Hurried

discussions at the AEC's headquarters, however, produced a list some seven hours after the regulations were made public.

AEC's "Mockery"

THE AEC's new environmental regulations sprung from a decision of the US appeals court on July 23, 1971, in which a group of environmentalist organizations accused the AEC of paying insufficient attention to the National Environmental Policy Act, particularly in regard to construction of a nuclear power plant on the Chesapeake Bay in Maryland. The following extracts are taken from the opinions of the appeals court:

The environmentalists charged the AEC with not submitting its detailed environmental impact statement to a review process unless it was specifically required to do so by public challenge. The court decided:

We believe that the Commission's crabbed interpretation of NEPA makes a mockery of the Act. What possible purpose could there be in the Section 102(2)(C) requirement (that the "detailed statement" accompany proposals through agency review processes) if "accompany" means no more than physical proximity—mandating no more than the physical act of passing certain folders and papers, unopened, to reviewing officials along with other folders and papers? What possible purpose could there be in requiring the "detailed statement" to be before hearing boards, if the boards are free to ignore entirely the contents of the statement? NEPA was meant to do more than regulate the flow of papers in the federal bureaucracy. The word "accompany" in Section 102(2)(C) must not be read so narrowly as to make the Act ludicrous. It must, rather, be read to indicate a congressional intent that environmental factors, as compiled in the "detailed statement", be *considered* through agency review processes.

On the AEC's decision not to consider environmental issues for permits issued before March 14, 1971, the court said:

Strangely, the Commission has principally relied on more pragmatic arguments. It seems an unfortunate affliction of large organizations to resist new procedures and to envision massive roadblocks to their adoption. Hence the Commission's talk of the need for an "orderly transition" to the NEPA procedures. It is difficult to credit the Commission's argument that several months were needed to work the consideration of environmental values into its review process. Before the enactment of NEPA, the Commission already had regulations requiring that hearings include health, safety and radiological matters. The introduction of environmental matters cannot have presented a radically unsettling problem. And, in any event, the obvious sense of urgency on the part of Congress should make clear that a transition, however "orderly", must proceed at a pace faster than a funeral procession.

Finally, the court instructed the commission to consider calling a halt to construction of some plants:

... In order that the pre-operating licence review be as effective as possible, the Commission should consider very seriously the requirement of a temporary halt in construction pending its review and the "backfitting" of technological innovations. For no action which might minimize environmental damage may be dismissed out of hand. Of course, final operation of the facility may be delayed thereby. But some delay is inherent whenever the NEPA consideration is conducted—whether before or at the licence proceedings. It is far more consistent with the purposes of the Act to delay operation at a stage where real environmental protection may come about than at a stage where corrective action may be so costly as to be impossible.

The new regulations place firmly in the hands of the AEC the responsibility for evaluating likely environmental effects of a nuclear power plant, including the so-called thermal pollution of cooling waters, and the commission is also charged with the task of balancing these likely effects with the growing need for electric power and with assessing alternative approaches. Environmentalists have in the past accused the AEC of being unresponsive to environmental questions because it has been in no position to evaluate alternatives, and therefore any attempt at a cost benefit approach to the licensing of a new power plant has been chiefly left to the electricity companies which cannot be expected to take a wholly unbiased attitude.

Environmental effects of proposed nuclear power plants will now be considered as a matter of course at two different stages of a licence procedure—when a company applies for a licence to construct a plant, and again when it applies for a licence to operate it. At the construction stage, the applicant must submit to the AEC a report spelling out in detail the environmental effects of the proposed plant, any adverse environmental effects that cannot be avoided should the proposal be implemented, alternative approaches to the proposed construction, "the relationship between local short-term uses of man's environment and the maintenance and enhancement of long-term productivity", and any irreversible and irretrievable commitment of resources which would be involved in the proposed action should it be implemented. The last two obscurely worded requirements are designed to help the AEC to conduct its own cost benefit analysis of the project, in terms of alternative methods for reducing environmental damage. An applicant for a licence to operate a nuclear plant must also submit to the AEC a report detailing the environmental effects of the plant, but only where they differ from the effects spelt out in the application for a licence to construct the plant.

The next stage in the process requires the AEC's Director of Regulations to draw up his own draft statement on the likely environmental effects of the plant, open the statement up for public comment, in the light of which he will draw up a final detailed statement and give his opinion on whether or not the permit should be issued. The detailed statement will include a cost benefit analysis balancing the environmental effects of the facility with its economic and technical effects.

The court of appeals chided the AEC for simply turning over the applicant's environmental report to other federal and state agencies, such as the agencies approved to regulate water quality standards under the Federal Water

Pollution Control Act, for assurance that the proposed plant will meet their standards. "The upshot is," the court pointed out, "that the NEPA procedures, viewed by the Commission as superfluous, will wither away in disuse, applied only to those environmental issues wholly unregulated by other federal, state, or regional bodies." This abdication of responsibility also prevented the AEC from considering the environmental effects of the proposed plant in the light of alternative actions. But the new regulations specifically require that the AEC's detailed statement includes a discussion of the effects "irrespective of whether a certification from the appropriate authority has been obtained".

The detailed environmental statement will then be offered in evidence if a public hearing is held, and the Atomic Safety and Licensing Board has been charged with the task of determining whether the requirements of the National Environmental Policy Act have been carried out in the proceedings. The licensing board will then have the honour of determining whether the licence should be granted, denied or modified to protect the environment. That decision would, of course, be open to public appeal, and all construction permits and operating licences would be issued on the condition that the licensee observes environmental conditions laid down by the federal government, the state in which it is to be built or operated and the Atomic Energy Commission.

The upshot of these new regulations is that the Atomic Energy Commission itself will be directly responsible for ensuring that the environment will not be abused by the construction or operation of a new plant, that environmental considerations are given prominence at every stage of the licence application, and that there is ample time for the public to offer a comment or a challenge on every report and decision made during the process. Another effect is that the regulations will add a further bottleneck to the processing of applications and place an extra burden on the commission's staff. Asked last week whether the regulations will slow up the granting of a licence which will permit operation or construction, Harold L. Price, the AEC's Director of Regulations, said that he hopes the present delay of eighteen months between application and granting of a construction licence will not be extended. Others, particularly the electricity producers, were not so optimistic, however, and the spokesman for one company said last week that licensing delays are already placing an intolerable burden on the utilities in trying to meet the consumers' power needs. (The Federal Power Commission states, however, that licensing procedures caused delays to only eight out

of 114 major power plants that started producing electricity between 1966 and 1970.)

Of even greater concern to the electricity producers is the provision in the new regulations for submitting to a detailed environmental review all the plants at present under construction or which have received operating licences since January 1, 1970, the date on which the National Environmental Policy Act came into force. Not only will these plants be reviewed, but the AEC is also asking that construction or operation be stopped until the review is completed. The electricity companies have been given thirty days to show why construction or operation should not be stopped before the AEC throws its powerful spanner in the works, and their only hope is to be able to show that continued construction or operation will not damage the environment significantly, that it will not preclude the adoption of environmental safeguards should they be necessary, and that delay would damage the public interest by failure to meet power demands or by causing undue costs to the licensee or the consumer. Five nuclear power stations that are already producing electricity and forty-six others that are under construction are threatened by this provision. Environmental reports must be submitted to the AEC detailing the environmental impact of the plant, and the Director of Regulations of the AEC must then go through the complete procedure of issuing a draft and later a final detailed environmental report on the plant. It is then up to the Director of Regulations to decide whether the licence or permit should be continued, modified to protect the environment, or be revoked, and his decision will be open to challenge in a public hearing.

In backtracking so vigorously on its past regulatory policies, the AEC has opened itself wide to court action from disgruntled electricity producers, and the sharpening of knives could clearly be heard in the industry last week. A spokesman for the Consolidated Edison Company, although reserving his comments until the company has had a chance to study the regulations in greater depth, accused the AEC of plunging the electricity industry into a crisis, and he pointed out that several electricity companies are intending to appeal against the appeals court decision which precipitated the AEC's new environmentally conscious image. Even if that appeal is successful, however, it is unlikely to affect the new regulations, although it would place the AEC in the embarrassing position of enforcing regulations that would no longer be upheld by a court decision, and this would serve to strengthen the hand of the electricity companies in any future court skirmish with the commission.

Of more immediate concern to the AEC, however, is the possibility that electricity companies will file a suit for losses caused by construction or licensing delays, or for the installation of expensive equipment such as cooling towers. In that case, the AEC would undoubtedly be on very sticky legal ground, for the companies have all proceeded under licences or permits issued legally by the AEC under its old regulations, but they now face the possibility of having those licences modified or revoked because the commission now regards them as inadequate for the protection of the environment. The AEC's policy switch has therefore put both the commission itself, and the industries it regulates, in an extraordinary position, but while the courts are being kept busy, environmentalists can take heart that the new chairman of the commission is not deaf to their pleas.

EMPLOYMENT

Unemployed PhDs

by our Washington Correspondent

MORE evidence about the tight job market for those holding a PhD in science has been published this week by the National Research Council. A survey, conducted between December 1970 and March 1971, of scientists who received their PhD degree in 1970, showed that 1.6 per cent were out of work, and that a further 1.2 per cent held jobs for which their academic training was unnecessary. Moreover, there are signs that many more PhDs are looking towards postdoctoral training as an escape from the realities of the job market, but the indications are that they will fare no better when they finally leave the groves of academe, for the National Research Council has discovered that of the PhDs that had been engaged in postdoctoral training

in 1970, 2.2 per cent were unemployed in the following winter and 0.9 per cent were employed in an "inappropriate position".

The results of the survey, which in many respects bear out the findings of an employment analysis conducted by the National Science Foundation (*Nature*, 232, 150; 1971), are a clear indication that the prospect of a dire shortage of PhDs that was predicted for the 1970s has not materialized, and that the universities are already turning out more PhDs than the market can absorb. The Office of Education, for example, predicted in 1964 that there would be a desperate shortage of PhDs by 1974, and a great drive was undertaken in the universities to make up the predicted shortfall. Last year, for example, 29,436 scientists received PhDs—an increase of 14 per cent compared with the previous year. Although, compared with the national unemployment rate, which has been running at about 6 per cent for the first half of 1971, the unemployment rate for PhDs is small, prospects for the rest of the decade look grim. The 1970 figure, for example, showed that the number of scientist PhDs out of work is increasing at an alarming rate (the 1970 figure was double the 1969 total), and the NSF has recently predicted that by 1980, at present rates, there will be about 45,000 more PhDs in America than there are suitable jobs.

The NRC survey has also brought out the fact that physicists are the worst hit single group of scientists, with 2.9 per cent of the PhDs and 3.3 per cent of the postdoctorals out of a job. Mathematicians, geoscientists and social scientists, on the other hand, reported lower than average unemployment rates, with 1.2, 0.7 and 0.5 per cent of their PhDs out of work in 1970. Even these subjects, however, reported greater unemployment rates for 1971 than for the

previous year. There was also a sharp increase in the number of foreign-born scientists unemployed in the United States—the figure rose from 0.9 per cent in 1969 (the same as for US citizens) to 2.3 per cent in 1971 (compared with 1.6 per cent for US nationals)—a figure that can only be partly accounted for by the fact that fewer foreign-born holders of the PhD left after completing their academic training in 1971 than in 1969.

One of the most significant findings in the survey is that the number of new recipients of the PhD who went on to postdoctoral work in the universities in 1970 increased by 4.5 per cent over the 1969 figure. This fact undoubtedly hides much of the unemployment, and disillusionment among PhDs fresh from the university production line, but with few prospects of appropriate employment outside the universities. Moreover, the situation will probably be made considerably worse by the fact that predictions made two years ago by the Office of Education, that graduate enrolment would have risen by 99 per cent by 1980, now seem to be vast overestimates—the NSF, for example, now predicts that the expansion may be nearer 45 per cent—thereby reducing drastically the predicted number of jobs for PhDs in the universities themselves.

The National Research Council itself concludes from the survey that "various market accommodations are undoubtedly responsible for preventing what would otherwise be a gross oversupply". Nevertheless, with an eye clearly on the mandarins in the Office of Education, the NRC says "any deficit of jobs must ... be a matter of national concern, and reliance solely on market adjustments to an oversupply would be both callous and unwise. The large investment in graduate education and the valuable human resources at stake demand that any underemployment or unemployment of doctorate recipients be corrected".

Percentage Distribution, 1971 Activity of 1969 and 1970 Graduates, by Citizenship, by Gross Field Categories

1971 activity	Citizenship	1969 graduates				1970 graduates			
		EMP*	Bio.	Soc. Sci.	Total	EMP	Bio.	Soc. Sci.	Total
Postdoctoral training	US	8.8	20.8	1.8	9.9	12.4	30.9	3.0	14.4
	Foreign	8.0	15.3	0.4	8.7	13.6	22.1	1.6	13.5
Appropriate jobs in USA	US	82.8	69.8	89.0	81.4	78.4	58.7	88.5	76.5
	Foreign	51.8	31.0	36.7	44.6	47.6	23.7	42.5	41.6
Employed in inappropriate work	US	0.6	1.0	0.4	0.6	1.3	1.2	0.8	1.2
	Foreign	1.3	0.4	0.0	0.9	1.3	2.6	0.8	1.5
Unemployed, total	US	0.7	1.1	1.0	0.9	1.4	1.6	1.2	1.4
	Foreign	0.8	1.3	0.4	0.9	2.7	2.0	0.8	2.3
Left US	US	1.6	2.5	2.4	2.0	2.0	2.7	2.3	2.2
	Foreign	28.8	48.8	54.9	37.4	30.5	44.2	48.5	36.4
All other	US	0.7	0.8	0.3	0.5	1.0	1.1	0.6	0.9
	Foreign	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0
Unknown	US	4.8	4.0	5.1	4.7	3.5	3.8	3.6	3.6
	Foreign	9.2	3.2	7.6	7.5	4.2	5.3	5.8	4.7
Total No. in sample	US	5,262	2,208	2,296	9,766	5,740	2,429	2,477	10,646
	Foreign	1,190	471	275	1,936	1,420	493	365	2,278

*EMP, Engineers, mathematicians and physicists.

NEWS AND VIEWS

Of Mice and Men

As the number of research workers setting their sights on the discovery of human tumour viruses increases—as it seems certain to do, especially in the United States where money for cancer research is becoming so easy to come by and money for anything else commensurately difficult to find—it can confidently be anticipated that there will be numerous repetitions of the sequel of events which has followed the report by Priori, Dmochowski and their colleagues (*Nature New Biology*, **232**, 61; 1971) that a cell line from a child with Burkitt's lymphoma produces a C-type RNA virus and which has culminated in the report by Gilden, Parks, Huebner and Todaro (see page 102 of this issue of *Nature*) that this is probably a mouse virus, presumably a contaminant.

Dividing cells, whether in an animal or maintained *in vitro* in cultures, provide an ideal environment for the replication of all sorts of viruses which have nothing whatsoever to do with malignancy. Any virus which by chance reaches a population of such cells is likely to become permanently established in it. And those biologists who are now turning to tumour virology, if only to keep themselves in a job, would do well to remember that whenever they find a virus in a fresh biopsy of tissue from a tumour or in a population of tumour cells in culture, the first priority is not to call a press conference but to recognize that the virus is probably a contaminant. As recent events seem to have shown, even such old hands in the game as Priori and Dmochowski can be tripped over by contamination, and their story is salutary.

Priori *et al.* established a monolayer cell culture from the pleural effusion of an American child with Burkitt's lymphoma. During the tenth passage of these cells they were found to bud typical C-type particles with all the morphological characteristics of the avian and animal RNA tumour viruses. Well aware of the problem of contamination, they apparently sent samples of their virus to Dr Old's group at the Sloan-Kettering Institute to have them classified by serological tests. The crucial question was simply does this virus have the group-specific antigen, which indicates species specificity, of any of the known animal sarcoma and leukaemia viruses or does it have a unique group-specific antigen which would strongly suggest that it is indeed a human C-type virus? Although Priori *et al.* gave few details of the tests performed in Old's laboratory they reported that their virus seemed to have a group-specific antigen different from any of the antigens of the known animal leukaemia viruses. Not surprisingly this result encouraged them to believe that they might well have isolated a human C-type virus, but both this result and its interpretation have now been seriously challenged by Gilden *et al.*

Gilden *et al.* obtained from Priori and Dmochowski some of the virus producing cells at their forty-first passage and taking every precaution to prevent any contamination of the cells in their two laboratories, they performed three sorts of immunochemical tests to see whether the virus was related to any of the known animal C-type viruses. The results of both complement fixation and radioimmunoprecipitin inhibition tests strongly suggested that something in the human, virus-producing

cells carried the group-specific antigens of the mouse leukaemia viruses. Pursuing this lead, they then performed immunodiffusion tests with sera specific for the mouse virus group-specific antigen and sure enough found a precipitin line of identity between highly purified murine group-specific antigen and concentrates containing the virus liberated by the human cells. In short, these results must mean either that the virus being produced by Priori and Dmochowski's cells is murine leukaemia virus, which presumably somehow contaminated the cultures, or mouse and human C-type viruses have group-specific antigens which cannot be differentiated by these tests. This second alternative seems to be most unlikely and, if the results of Gilden *et al.* can be independently confirmed, it will be hard to avoid the conclusion that the virus Priori *et al.* have isolated is anything other than a contaminant and a red herring.

But as one putative human cancer virus is eliminated, others are turned up. The two virus-like particles from cultures of cells from patients with Hodgkin's disease, which Kingsley Sanders's group describe on page 104 of this issue of *Nature*, for example, must be suspect until they have been further characterized. Perhaps stimulated by the fascinating epidemiological study of a rash of Hodgkin's disease cases among the 1954 graduating class of a New York State high school, which lead Vianna, Greenwald and Davie (*Lancet*, i, 1209; 1971) to conclude that Hodgkin's may well be an infective disease with a long incubation period and a carrier state, Kingsley Sanders and his colleagues established a number of long-term cell cultures from lymph node material of Hodgkin's patients and searched for virus particles. The cells in the cultures underwent some bizarre changes including a "blastoid transformation" during which "practically every reticular cell could be seen to have one or more round cells within it", but they also began producing two sorts of virus-like particles. One of these is apparently a DNA containing particle which under the electron microscope resembles a herpes virus and there is evidence that the cells after the blastoid transformation have herpes virus antigens. The second particle which has so far defied all but the most preliminary characterization apparently contains RNA but under the electron microscope nothing with the characteristic morphology of a virus has yet been seen.

Obviously these observations are extremely preliminary; whether or not these cells are producing an RNA virus remains to be seen and the significance of the herpes virus is at present anybody's guess. Nevertheless, the findings of Vianna *et al.* strongly suggest that a virus which plays a part in the aetiology of Hodgkin's disease awaits discovery and it can be certain that Kingsley Sanders and his colleagues will soon find themselves in a crowded field, for if so many workers are set on searching for human cancer viruses they may as well concentrate on one of the extremely few forms of human cancer for which there is at least some epidemiological evidence to suggest the existence of a virus. And it scarcely needs adding that a more extended investigation of the epidemiology of Hodgkin's disease would not come amiss.

CANCERS

Dangers of Affluence

from our Cell Biology Correspondent

THE fig and prune producers of the world might do worse than start an advertising campaign with the slogan "a bowl a day keeps bowel cancer away" to judge from what Burkitt has to say in the current issue of *Cancer* (28, 3; 1971). Burkitt has, of course, spent many a long year trying to pin down environmental factors responsible for particular cancers, always arguing that the incidence of the disease can be significantly reduced more easily by eliminating or evading the causative agents than by trying to fathom out their mode of action and devise a cure. And his latest foray, this time into the epidemiology of cancer of the colon and rectum, leads him to the conclusion that the refined diets of the world's developed nations, in particular their lack of indigestible fibre, may, together with particular gut floras, be responsible for the comparatively high incidence of bowel cancers and other non-malignant and non-infectious diseases of the bowel in affluent societies.

The incidence of bowel cancer, which in most western countries is second only to the incidence of lung cancer, is correlated with the stage of economic development, and Burkitt claims that the age adjusted incidence of this disease is ten-fold greater in Western Europe and North America than in most African countries. Epidemiologists when faced with such findings frequently resort to the incantation "dietary differences" and leave it at that with the real problem, the identification of the crucial dietary components, unsolved. Burkitt to his credit has given hostages to fortune by suggesting what the crucial factors may be, namely the transit time of the stools through the bowel and the nature of the bowel flora.

By recruiting the help of African and English boarding school boys, who were provided with radio-opaque plastic pellets as a condiment for their school meals, he and his collaborators have shown that there is a clear inverse relationship between the transit time and bulk of the stools and the fibre content of the food eaten—the more fibre the shorter the transit time. African boys passed 80 per cent of the plastic pellets in 45 h whereas their English counterparts took 89 h. Burkitt suggests therefore that carcinogens produced by the bacterial flora in the bowels of westerners, whose foetid stools contrast markedly with the odourless stools of Africans, are held at high concentrations for prolonged periods in contact with the bowel

mucosa with the net result of an increased incidence of bowel cancer in western populations. Like a prophet in the wilderness Burkitt writes: "In view of the evidence, it seems justifiable to issue a warning against the removal of so much of the unadsorbable fibre from our food, and the associated over ingestion of refined carbohydrate".

Another and related warning was published recently by Hill, Goddard and Williams (*Lancet*, i, 472; 1971) who suggest that differences in fat consumption together with differences in gut flora may account for the geographical variations in the incidence of breast cancer. It is well known that mammary carcinoma is far more prevalent among North American and North West European women than among African, Asian and South American women, and surveys of the Japanese populations in Japan, Hawaii and the West Coast of the United States show that differences in the incidence of breast cancer depend on geographic rather than genetic factors. Dietary differences

have been discussed, and Wynder, for example, has postulated that the consumption of large amounts of fat may affect hormone production and/or storage in adipose tissue and thus be responsible for an increased incidence of breast cancer.

Hill and his colleagues now suggest an alternative mechanism. They have shown that people eating high fat diets have in their guts more active and strictly anaerobic bacteria than people eating low fat diets. Moreover these bacteria are able to produce oestradiol, oestrone and several other steroid sex hormones. If one or more of these steroids is carcinogenic or acts synergistically and stimulates the growth of tumour cells, anaerobic gut bacteria may be the link between high fat diets and a high incidence of breast cancer, and for that matter colonic cancer. If the much discussed expansion of cancer research materializes, the distributors of the dollars might do worse than promote further investigations of the metabolic activities of gut bacteria.

Liquid Drops in Shock Tubes

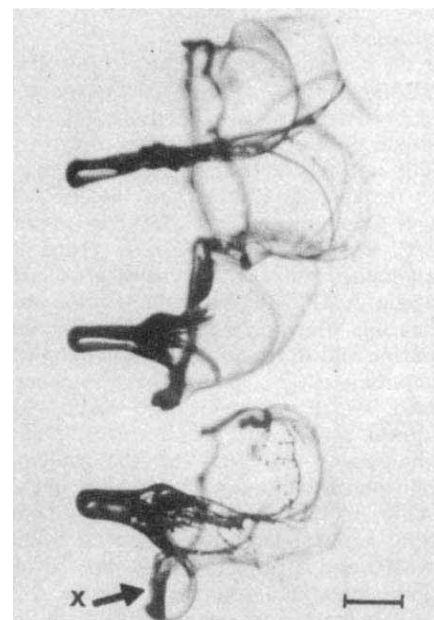
THE photograph below shows some of the bizarre shapes that drops of water can take up in a shock tube. The drops are falling through a flow that is from left to right, and the aerodynamic and surface tension forces involved have given the drops an umbrella-shaped canopy. The photograph is one of several in next Monday's *Nature Physical Science* that have been obtained by P. G. Simpkins of Bell Telephone Laboratories, Whippany, New Jersey, illustrating the successive deformations that drops undergo when exposed to a sudden increase in the external flow.

The crucial parameter in work of this nature is the ratio of the force due to aerodynamic pressure to the force due to surface tension—the Weber number—and the photograph was taken at a Weber number of 45. But the region in which Simpkins is interested has a lower limit at a Weber number of about 7.3—above this critical value the non-uniform distribution of surface pressure exerted on the drops causes deformations which eventually lead to the bursting of the drops.

The steps in this process, as charted by Simpkins, are as follows. At a Weber number of 19 drops that initially had a diameter of 1 mm are flattened by the flow into thin disks. The centres of the disks then blow out in the direction of the external flow, so that the drop takes on the appearance of a bag supported by a liquid annulus. Simpkins presents graphs showing how the distortion proceeds, and discusses the deviations in behaviour from those

predicted by simple theory.

The deviations from expected behaviour become more severe at higher Weber numbers. Umbrella-shaped distortion of the drops like that in the photograph occurs at Weber numbers above twenty. The mechanism by which the drops form a narrow stem and subsequently a canopy is, however, unknown.



Drops in a shock tube at a Weber number of 45, 1,660 μ s after the start of the flow. The flow is from left to right and the bar represents 1 mm. The arrow points to a satellite drop which is showing the bag response.

PROTEINS

The Mighty Photon

from our Molecular Biology Correspondent

THE use of the photon as a reagent, or as a trigger that will set off other reagents at a chosen point in space or time, is an approach that will undoubtedly find increasingly wide application in molecular biology. One—albeit qualified—advantage is that short-lived photochemically generated species are inclined to have very high reactivity, and when unleashed on an enzyme, for example, are apt to react even with aliphatic side chains. Too indiscriminate a reactivity of course restricts the value of a reagent, and Sperling and Elad (*J. Amer. Chem. Soc.*, **93**, 3839; 1971) have addressed themselves to ways of controlling the specificity of photochemical alkylation. They have found that on irradiation of a system containing a peptide, an unsaturated species, such as butene or toluene, and an initiator, substitution occurs at the α -carbon of glycine residues, butene giving rise to norleucine, toluene to phenylalanine. When proteins are allowed to react under these conditions, however, histidine, cystine, methionine, tyrosine and tryptophan residues also react. This is a consequence of the formation of various free radicals, and if radical scavengers are added to the reaction mixture the majority of these side reactions are suppressed. It proved impossible, however, to prevent instant death to the tryptophans. In ribonuclease, which has no tryptophan, glycine reacted and, though a certain amount of other damage occurred, 20 per cent of enzymatic activity was retained.

Of more immediate biological moment is the design of photochemically triggered affinity labels, which hold out the advantage that they will not react fruitlessly on their way to the specific binding site. Brunswick and Cooperman (*Proc. US Nat. Acad. Sci.*, **68**, 1801; 1971) have prepared analogues of the biochemical elixir, cyclic AMP, labelled with tritium and bearing diazomalomyl groups in the purine and in the sugar. On irradiation a ferociously reactive carbene is generated, which is expected to react with almost any group within reach. The analogues will bind to the enzyme phosphofructokinase, for which cyclic AMP is the natural activator. They both activate and compete with cyclic AMP, and after irradiation are covalently integrated into the enzyme.

This work now opens the way to the isolation of a labelled peptide and the characterization of the activator binding site. A more exciting prospect for the biochemist, however, is the use of the same reagents to label cyclic AMP

receptors in cells, and indeed Brunswick and Cooperman have already succeeded in introducing a label into a protein component of rat testis. The reagent is thus permitted to traverse the membrane and diffuse through the cell to its target protein before it is irradiated and caused to react. The advantages of this strategy in labelling components in intact tissue need no labouring.

Erlanger and his colleagues have meanwhile continued their attempts to simulate the photochemical systems of nature in the laboratory. By the use of acetylcholine analogues capable of light-induced *cis-trans* isomerization they achieved partial control over the activity of an excitable tissue preparation, because the isomers will not in general be expected to possess precisely the same binding characteristics. Bartels, Wassermann and Erlanger (*ibid.*, 1820) have now brought this essay in biochemical engineering to a considerable state of refinement. They have, apparently by a process of serendipity, arrived at a species—an azobenzene derivative—of which the *trans* isomer is one of the most powerful known activators, and the *cis* isomer is largely, perhaps completely, inactive. They have moreover built into such a

molecule the means for attaching it covalently to its receptor. This is based on the earlier observation by Karlin that affinity labelling of the acetylcholine receptor of the electroplax membrane could be achieved with a thiol-reactive function, if the tissue was first treated with a reducing agent to break a disulphide bond, evidently located hard against the binding site.

Substantial *cis-trans* interconversion of the azobenzene derivative can be achieved by ultraviolet irradiation, and is reversed by white light. Thus when the system containing electroplax tissue, stimulated by the *trans* isomer, is exposed to the near-ultraviolet light, precipitous depolarization ensues. Competition with carbamylcholine and curare occurs as expected. When the thiolreactive version of the analogue is covalently attached to the reduced membrane, the competitive effect of carbamylcholine is lost. This then is a completely self-sufficient light-regulated system. The reactive *trans* isomer is reported to inhibit acetylcholinesterase, but so equally does the *cis* form. The inferred message is that the acetylcholine receptor and acetylcholinesterase are distinct and unrelated forms.

Plasma Current Amplifier

IN next Monday's *Nature Physical Science*, P. C. Stangeby and J. E. Allen describe a new device which contains a narrow metal-walled electric discharge vessel and which is capable of high current amplification. The metal walls behave as a cathode and electrons are extracted from the metal by surface recombination with ions that arrive from the plasma.

According to the theoretical description of the device, the ion current travelling to the walls of the conducting cylinder is related to the discharge arc current in an expression involving the radius of the cylinder, the electron and ion masses and other constants which are of the order of unity. The cylinder is biased in such a way that the electrons produced by ionization of the neutral particles which carry them away from the wall are retained in the arc discharge; the ions can then return to the cylinder to pick up other electrons. The plasma current thus increases exponentially with distance down the cylinder and, for small cylinder radii and small ion masses, the multiplication factor is very large (for H_2 in a 5 mm diameter cylinder, for example, a current amplification of about 10^7 can be obtained).

The prototype apparatus used by Stangeby and Allen comprised a metal

tube 6 mm in diameter and an anode which could be moved inside the tube. There were two alternative cathodes—a mercury pool type and a thermionic type. A 2 A current from the mercury pool cathode was made to flow across the mouth of the metal discharge tube and was collected by an auxiliary anode; the required current input to the metal tube itself was then tapped off.

The thermionic cathode gave similar experimental results, but one of its advantages was that the tube current could be measured directly with an ammeter. There was also a facility for introducing a quantity of argon gas to examine the effect of reducing the ion mass (which should increase the amplification). Measurements with the apparatus have shown that the current amplification at a mercury pressure of 1 mm Hg and an argon pressure a few hundred times this value is as expected theoretically. Although they did not measure the argon flow rate accurately, Stangeby and Allen found that an increased rate was associated with a distinct increase in the amplification.

There are probably several uses for current amplifying devices of this type, including applications to high power lasers; they may also be useful as high current controls.

PERMO-TRIASSIC

Problems left Unsolved

from a Correspondent

AN international conference, with problems of the Permian-Triassic boundary as its principal theme, was held from August 23-26 at the University of Calgary, Alberta, Canada. The keynote of the conference was set by Dr A. McGugan, speaking on behalf of Dr N. D. Newell (American Museum of Natural History, New York), who outlined some of the major problems of the late Permian and early Triassic. These included the almost universal occurrence of discontinuities or paraconformities at or near the system boundary, the general poverty of the contemporary fauna and the wide extent of essentially barren continental red beds.

The concept of a universal hiatus in marine strata at or near the system boundary was emphatically supported by Dr E. T. Tozer (Geological Survey of Canada, Ottawa), and, although there was some disagreement on exact placing, it was generally conceded that a break is present in each of the classic mid-Tethyan sequences of Armenia, Kashmir and the Salt Range of West Pakistan. The duration of the hiatus was thought on some palaeontological evidence to be of the order of one to two stages, but Dr W. G. Sweet (Ohio State University) considered that the break could not be recognized in conodont assemblages. In an unscheduled and characteristically forthright paper, Dr R. Assereto (University of Milan) asserted that the hiatus might have been shorter in some eastern parts of the southern Alps than in the classic sections farther east, and Dr H. Taraz (Geological Survey of Iran), whose view was challenged in discussion, suggested that no breaks were present in a thick late Permian and early Triassic marine sequence in central Iran. He proposed a loosely defined Abadeh stage for the time represented by post Djulfian pre-Triassic strata.

The staggered but progressive decline in late Permian floras and faunas was documented by several speakers, including Drs R. Toryama (Kyushu University) and G. L. Wilde (Humble Oil and Refining Company, Houston) who described the final stages in the evolution of the fusulinids, and Dr G. L. Batten (American Museum of Natural History, New York) who showed how a dramatic decrease in marine gastropod genera took place in the late Permian and was not fully recouped until the Ladinian. Among plants, Dr J. M. Schopf (US Geological Survey, Columbus) demonstrated a sharp unexplained change from the dominant *Glossopteris* flora of the Permian to a Triassic flora dominated by *Dicroidium*.

Several speakers speculated on the cause of the floral and faunal decline in the late Permian, without reaching agreement. Drs J. Pattison, D. B. Smith and G. Warrington (Institute of Geological Sciences, Leeds) suggested that a major cause might be the virtual elimination of shelf seas, during a prolonged phase of tectonic stability and peneplanation following rapid build-up of evaporites and progradation of coastal plain sediments. This view was supported by Dr B. Waugh (University of Hull) and, with variations, by several other speakers. There were tentative suggestions that the changes in the contemporary biota might have been related to the beginning of the break-up of Laurasia, and Dr G. F. Stehli (Case Western Reserve University) showed that seafloor spreading could be reconciled with the distribution of known Permian and Triassic biotas as well as that of evaporites and red beds.

The effects of late Permian and early

Triassic plate movements along the Pacific margin of the United States were graphically interpreted by Dr N. J. Silberling (Stanford University), but Dr A. A. Meyerhoff (Tulsa), opposing the concept of continental movements, claimed that the biotic crisis could have been caused by cosmic ray and temperature maxima associated with rare galactic events of a type calculated to have prevailed near the end of the Permian. Finally, the view that late Permian floras and faunas might have declined following nutrient depletion and a progressive failure of lower members of the food chain was persuasively argued by Drs Helen Tappan and A. R. Loeblich (University of California).

In the closing sentence of his introduction, Dr N. D. Newell had expressed the wish that the conference would leave unsolved at least some of the Permian and Triassic problems: his wishes, perhaps unwittingly, were duly gratified.

How SV40 DNA Replicates

THE mechanism of replication of such covalently closed circular DNA molecules as the chromosomes of *Escherichia coli* and the coliphages lambda and ϕ X 174 has long been a source of fascination to molecular biologists. And there continue to be arguments for and against the Cairns's model of replication, which predicts that none of the DNA strands in the replicating structure is longer than a parental strand and the two progeny strands are of equal length, and the rolling circle model, which predicts the occurrence of some DNA longer than parental strands and progeny strands of unequal length. The replication of the genomes of the DNA tumour viruses SV40 and polyoma virus, which are not only closed, circular, double stranded DNAs but are also supercoiled, presents, of course, the same topological problems as the replication of circular phage and bacterial DNAs. And the experiments published in *Nature New Biology* next week by Jaenisch, Mayer and Levine clearly indicate that it is by the Cairns's mechanism that these molecules duplicate.

Jaenisch and his colleagues used the Hirt procedure to extract preferentially the SV40 DNA from infected African green monkey kidney cells, which 40 h after infection had been exposed to ^3H -thymidine for periods ranging from 3.5 min to 120 min. They then analysed the viral DNA on sucrose, ethidium bromide-caesium chloride and alkaline gradients and examined it by electron microscopy. In summary they find that replicating SV40 molecules contain parental closed circular DNA strands as well as linear fragments of

newly made DNA. Moreover, the DNA labelled during the shorter pulses is shorter than one complete SV40 strand and is not linked to parental strands; the rolling circle model, of course, predicts the opposite of these findings.

Molecules which are partially (10-70 per cent) replicated sediment faster than anticipated, but under the electron microscope the reason for this becomes obvious; these molecules have two untwisted branches of equal length attached to a third twisted or supercoiled branch and, as expected, the length of one untwisted strand plus the twisted strand is about the same as the length of a single relaxed SV40 genome. The electron microscope also reveals single stranded discontinuities at the forks and some 34 per cent of the molecules examined have these regions at both forks. If a single stranded region at a fork indicates a site of replication then at least some SV40 DNA molecules must be replicated in two directions from an origin, although this cannot be obligatory, for 41 per cent of molecules had only one fork with single stranded DNA.

By showing in reconstruction experiments that relaxed SV40 DNA molecules are not converted into covalently closed, double stranded circles during the experimental procedure, Jaenisch *et al.* have disarmed critics who might have suggested that *in vivo* the template for replication is a nicked DNA strand, which, during the experiment, is closed into a ring. And so it is hard to escape the conclusion that at least some SV40 DNA molecules replicate by the Cairns's mechanism.

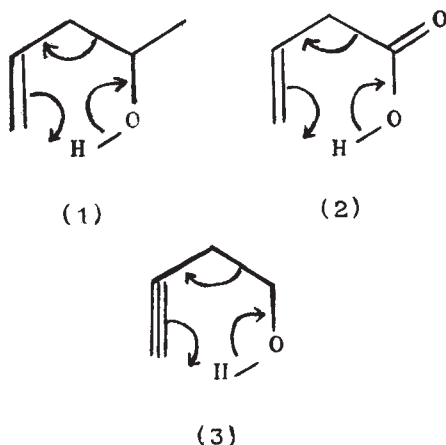
REACTION MECHANISMS

Where is the Proton?

from our Biological Chemistry Correspondent

KINETIC isotopic effects provide a specialized tool for investigating transition states of reactions which involve slow proton transfers. At the qualitative level, the technique of exchanging hydrogen for deuterium has many successes to its credit, not least in the field of enzymatic mechanisms. The problems emerge when attention is directed to the significance, if any, of the quantitative aspects of primary isotopic effects.

The early theoretical appraisals, notably due to Westheimer and to Biegeleisen, developed the concept that the magnitude of the kinetic change observed on isotopic substitution should reflect the extent of hydrogen transfer in the transition state: "maximum" effects correspond to those reactions in which the hydrogen is symmetrically located between the donor and acceptor groups in the transition state. Although this idea has been widely applied, direct supporting data have been sparse and the concept has been challenged often, most recently by F. G. Bordwell and W. J. Boyle (*J. Amer. Chem. Soc.*, **93**, 512; 1971) who searched in vain for a simple correlation between isotopic effect and extent of proton transfer.



H. C. Kwart and M. C. Latimore (*ibid.*, **93**, 3770; 1971) have now provided strong validation of Westheimer's concept by investigating three gas-phase reactions. The thermolytic fragmentation of 4-penten-1-ol (1), 3-butenic acid (2), and 3-buten-1-ol (3) all proceed through concerted bond-reorganization processes which unquestionably involve transfer of hydrogen from oxygen to carbon—formally indicated by the "curly" arrows, but probably sigmatropic processes. In spite of this similarity, the three cases provide a significant variation both of the acidity of the hydrogen being transferred and in the electronic

structure around the acceptor-carbon. If therefore these factors influence the isotopic effect, differences should become apparent.

The rates of all three reactions, measured over a range of temperatures, show a 2-fold to 2.5-fold rate retardation on replacement of hydrogen by deuterium. "Maximum" values for the isotopic effects were calculated on the assumption that the zero-point energy difference, O-H vs O-D, alone determines the kinetic isotope effect. In each case, observation and calculation agree within the limits of experimental error.

These results thus provide one of the strongest supports for the continued use of kinetic deuterium isotopic effects as a criterion for concertedness in hydrogen transfer reactions.

HEPATITIS

Active Immunization

from our Medical Virology Correspondent

IT has been repeatedly demonstrated in many parts of the world that the injection of normal human immunoglobulin (gamma-globulin) during the incubation period of infectious (epidemic) hepatitis can prevent jaundice among contacts. Although the incidence of the icteric form of hepatitis was thus reduced, infection was not prevented—it was attenuated to a variety of hepatitis without jaundice or to the subclinical form of the disease and faecal excretion of the virus still occurred.

The precise mode of action of

immunoglobulin is not completely known but it is presumed that partial passive, and therefore short term, immunity against infectious hepatitis is obtained by the antibodies present in the globulin prepared from large pools of plasma. Active immunity may, of course, be acquired through subclinical infection, so-called passive-active immunity.

The problem with serum hepatitis, on the other hand, seems to be entirely different, and immunoglobulin has not reduced significantly the incidence of post-transfusion hepatitis. The discovery of the association between Australia antigen and serum hepatitis has provided the crucial key for studies of this form of hepatitis. S. Krugman and his associates (*J. Amer. Med. Ass.*, **217**, 41; 1971) now report the results of limited studies in volunteer children on active immunization against serum hepatitis caused by the MS-2 strain. Thirty-nine children were divided into two groups: one group of twenty-five susceptible children were injected with MS-2 serum; the second group included fourteen susceptible children who received either one or two inoculations of heat inactivated preparation of a 1 in 10 dilution of MS-2 serum followed by an inoculation of untreated infective serum 4 to 8 months later. It was found that hepatitis induced by MS-2 serum was prevented by active immunization and that two spaced inoculations were more effective than one. Nevertheless, one inoculation gave enough protection to prevent some cases of serum hepatitis and to modify others. Krugman and his colleagues emphasize that these obser-

"Unblocking" Sera in Anti-tumour Immunity

DURING the past few years evidence has accumulated that animals and human patients with progressively growing tumours often have immune cells that are capable of reacting against their own tumour cells, as shown by cytotoxicity tests *in vitro*, but they also have in their sera factors that can specifically block the anti-tumour reactions. To increase anti-tumour immunity it would be useful if ways could be found for overcoming such blocking effects. The possibility that another group of serum factors can "unblock" immune reactions against tumours arises from the work of the Hellströms and their associates on sera from mice with regressing Moloney sarcomas, and of Bansal and Sjögren on polyoma virus-induced tumours in inbred rats. The latest report from these last authors will appear in next Wednesday's *Nature New Biology*.

Bansal and Sjögren have found that when sera from rats immune to polyoma tumours are mixed with homologous

blocking serum, they counteract the inhibition produced by the blocking serum on lymphocyte-mediated cytotoxicity against polyoma tumour cells *in vitro*. Still more remarkable, such sera inoculated into five rats bearing transplanted polyoma tumours brought about regression of the tumours in four of the animals. These effects are not caused by anti-viral antibody, and it seems unlikely that they are caused by serum-mediated autotoxicity because the transplanted cells grew into palpable tumours before regressing—such regression is never seen in untreated rats. Further work is required to establish how common unblocking activity in serum is, whether it is caused by a particular kind of antibody, and how it unblocks. One interesting possibility is that blocking is caused by antigen-antibody complexes, with which unblocking could react. Whatever the mechanism, there is no doubt that unblocking is of general interest as well as potential therapeutic significance.

vations are based on relatively small numbers.

These studies represent the culmination of many years of research using human volunteers. The use of mentally handicapped children and prisoners in such studies has been criticized on many occasions and the background and justification for such studies were recently summarized by S. Krugman and J. P. Giles (*J. Amer. Med. Ass.*, **212**, 1019; 1970). Apart from the ethical issues involved, however, several questions are inevitably raised by these studies. First, it is known that although the MS-2 serum contains Australia antigen this serum gives rise to a mild and now well characterized form of serum hepatitis. Second, the agent of serum hepatitis has not been finally characterized and the injection of heated and diluted whole serum containing Australia antigen is rather a crude way of inducing immunity. The serum may, of course, be regarded as an inactivated "vaccine", but some purified inactivated vaccines such as measles and respiratory syncytial virus have sensitized rather than protected the recipients. In terms of hepatitis this is an important problem because several workers have suggested that there are similarities between Australia antigen-associated hepatitis and type 3 hypersensitivity reactions in which the severity of the disease may depend on the balance between antigen and antibody (see review by A. J. Zuckerman, *Bull. Wld Hlth Org.*, **42**, 957; 1970). Finally, active immunization against serum hepatitis is likely to have only limited application, for example to children and adults in crowded institutions such as hospitals for the mentally handicapped and more so to certain groups at high risk to hepatitis such as patients and staff of renal dialysis units, transplant surgeons and perhaps some laboratory workers. The problem of active immunization against the much commoner epidemic form of hepatitis remains elusive.

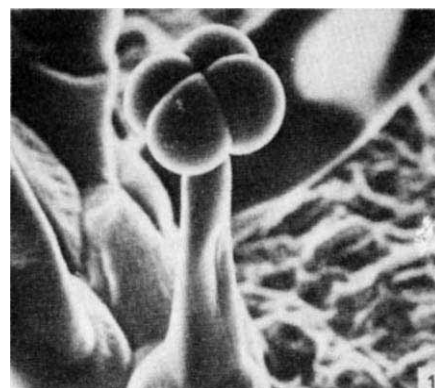
APHIDS

A Sticky End

SOME species of wild potato seem to have a most effective mechanism for resisting attack by aphids—the glandular hairs on the leaves and stems discharge a gummy substance when damaged by the tarsi of an aphid; this exudate first of all restricts the insect's movements and further accumulations lead to the complete immobilization of the aphid and to its death. This mechanism is described by Dr R. W. Gibson of Long Ashton Research Station, Bristol, who has been experimenting with three species of wild potato, *Solanum polyadenium*, *S. tarijense* and *S. berthaultii*,

and two aphid species, *Myzus persicae* and *Macrosiphum euphorbiae* (*Ann. Appl. Biol.*, **68**, 113; 1971).

In the three species of wild potato examined, the gummy exudate is produced from the tip of the glandular hair when an aphid mechanically ruptures the cell walls. In the intact hair, this substance seems to be a clear, water soluble liquid but on contact with air it changes to a dark brown or black insoluble sticky substance which is deposited on the aphid's limbs. Gibson found that not every contact of an aphid with a glandular hair resulted in the release of the exudate, but about 30 per cent of the aphids placed on any of the three species of wild potato became stuck to the plants by the substance within 24 hours. Starvation was probably the final cause of death; Gibson also noticed that the gummy substance occasionally found its way on to the labium of an aphid and this may also have prevented the aphids in his experiments from feeding.



Glandular hair on *Solanum berthaultii* of the type consisting of a four-celled head mounted on a stalk.

Although Gibson observed a wide variety of hairs on the leaves and stems of the three wild potatoes, the type with the four-lobed head (see figure) mounted on a stalk seems to be the principal, if not the only, source of the gummy material. These hairs are abundant on

Turning a Corner in Nucleic Acid Structural Studies

IN many ribonucleic acids, double helical base-paired regions are formed by a single polynucleotide chain folding back on itself. It is usually (and not unreasonably) assumed that these double helical regions have a structure similar to that determined by X-ray crystallography for the completely double helical molecules of reo virus and wound tumour virus RNA. But there have been no such impeccable guidelines for the conformation of the single stranded loops at the end of each double helix. Many of these loops, such as that containing the anticodon in transfer RNA, can be expected to have important roles in biological function and information on their structure is unquestionably of great significance. No doubt much will be revealed when the first single crystal analysis of a transfer RNA molecule is achieved. This happy event, however, still seems a little way off. In the interim attention has turned to the problem of turning a corner along a polynucleotide chain.

The information comes from the determination by Seeman *et al.* (see next Wednesday's *Nature New Biology*) of the crystal structure of the naturally occurring dinucleoside phosphate, uridine 3', 5' adenosine phosphate (UpA). There are, in fact, two molecules of the dinucleotide in each asymmetric unit in this crystal, thus providing at some modest additional effort to the authors two independent determinations of the structure of UpA. Although these two molecules have quite different overall conforma-

tions, Seeman *et al.* point out that these are attributable primarily to differences in orientation about only two single bonds—the two successive P-O bonds along the dinucleoside chain. The orientations found in one of the molecules are such that a polynucleotide chain containing a dinucleoside with such a conformation would turn a corner—actually through 180° in the space of one phospho-diester link. This structure observed for UpA seems to represent a particularly neat solution for the production of a sharp bend in a polynucleotide chain and it would be surprising if use is not made of this stereochemical possibility of some close variant of it in studies of the biological function of nucleic acid.

The interbase hydrogen bonding in UpA is also of considerable interest. Although base pairing is common in crystals of bases, it is relatively rare in crystals of nucleosides and nucleotides where hydrogen bonding of the bases to water, sugar hydroxyls and charged phosphate oxygens is frequently observed. This is a point which many builders of transfer RNA models seem not to have realized, because in most models tertiary structure is attributed to base pairing between single stranded regions of the clover leaf. In this context the structure of UpA is an exception to the rule because it does have base pairing. This, however, is not complementary pairing in which adenine-uracil pairs of the Watson-Crick type hold together two molecules of UpA, but is self-pairing between uracils and also between adenines.

all three wild species examined—they reach their greatest density on the stems—but are very scarce in the cultivated potato, *Solanum tuberosum*.

Could this resistance mechanism in wild potatoes be bred into the cultivated potato? Gibson provides no hint of the practical problems involved, but suggests that in theory this form of resistance in a strain could combine very successfully with resistance based on mechanisms which increase the restlessness of the aphids.

OCEANOGRAPHY

Changing Chemistry

from a Correspondent

IN connexion with the celebration of the 350th anniversary of the founding of Göteborg, the Nobel Foundation agreed to hold a symposium (the twentieth in the series) on the theme of the changing chemistry of the oceans with particular reference to the influence of man on the marine environment. This meeting took place at Aspenäsgränd near Göteborg on August 15–20.

The opening address was given by Professor Arne Tiselius (University of Uppsala), chairman of the Nobel Committee for Chemistry, and a Nobel prizewinner. He stressed the importance of marine science and requested that the delegates draw up a series of resolutions on aspects of marine pollution to be submitted for consideration at the United Nations conference on the human environment to be held in Stockholm next year.

Although many of the formal contributions, and much of the subsequent discussion, did revolve around various aspects of marine pollution, purely academic concepts were not neglected. The overall scope of the symposium included world-wide atmospheric and oceanic circulation patterns and their variability, trace gases in both the atmosphere and sea water, the chemistry of marine aerosols and surface films, bacterial activity in the oceans, the control of energy cycles in the sea, the impact of man on the major sedimentary cycles, the atmospheric transport of material to the oceans and the budget of trace solubles in sea water. The case histories of certain pollutants, such as mercury, chlorinated hydrocarbons (DDT and PCBs), phosphorus and hydrogen sulphide received special consideration.

Dr J. Ui (University of Tokyo) spoke of the dangers to health which may arise from the release of mercury into the natural waters of a densely populated area. He illustrated his talk with a harrowing film showing victims of the "Minimata" disease which was caused by consumption of fish contaminated by methylmercury formed bacterially

from inorganic mercury present in wastes discharged into Minimata Bay, Japan.

It was his opinion, which was endorsed by other speakers, that mercury pollution, terrible though its consequences are, is only likely to be a local problem, because the amount of mercury reaching the sea as a whole is only a small fraction of that entering by geological processes. He considered that a much greater threat is posed by toxic chlorinated hydrocarbons such as DDT and polychlorinated biphenyls (PCBs) which enter the sea by way of the atmosphere, and are therefore distributed globally. These lipophilic compounds are concentrated by marine animals at many levels in the food chain.

Dr Ui's arguments were reinforced by analyses presented by Professor E. D. Goldberg (Scripps Institution of Oceanography) and Dr V. T. Bowen (Wood's Hole Oceanographic Institution) which showed that concentrations of the PCBs in marine animals from both the Pacific and the Atlantic were often many times higher than those of DDT; values as great as 300 p.p.b. (fresh weight) of PCBs have been recorded for zooplankton from the

north Atlantic. It was suggested that concentrations of these chlorine-containing compounds are reaching the levels at which they may produce ecological effects, and that further increase may present a hazard to human health. It was the unanimous opinion of the symposium that restrictions on the use of these chemicals should be applied internationally.

Professor Goldberg discussed one of the most important concepts in contemporary environmental science—that man himself has now become an important geological force in his own right. He made a comparison between the fluxes of material introduced into the environment by man and those introduced by natural phenomena and concluded that man must now be considered a serious rival to nature.

Considerable time was spent on discussions about international measures which should be taken before it is too late to control and monitor the discharge of toxic, or potentially toxic, wastes into the sea. Emphasis was placed on the need for research into the ecological effects of the compounds concerned, and for studies of their routes through the marine food chain, and of the way in which they are degraded.

How *lac* Repressor Works

IN next Wednesday's *Nature New Biology*, Pastan and his colleagues report their latest manipulations of their cell free system which allows them to observe *in vitro* the regulated expression of the lactose operon (see *Nature New Biology*, **231**, 139; 1971). They have now focused their attention on the question of how the *lac* repressor prevents transcription, and have come to the conclusion that with the wild type *lac* operon the repressor does not prevent the actual binding of RNA polymerase to the promoter site but somehow prevents the bound polymerase from getting on with the job of transcription. But with a mutated *lac* operon, which carries a mutation in the promoter site that enhances the expression of the operon *in vitro* as well as *in vivo*, the repressor acts in part by preventing the binding of polymerase.

Pastan and his colleagues incubated wild type *lac* DNA with RNA polymerase in the presence of the cyclic AMP binding protein and cyclic AMP, which they believe somehow enhances the affinity of the polymerase for the promoter region. The polymerase as a result binds to the promoter and in the absence of repressor and the presence of the nucleoside triphosphates it can transcribe the operon. If, however, Pastan *et al.* incubate the DNA with repressor either before or after it is incubated with the polymerase, tran-

scription is blocked. Moreover, if an inducer is added to the system, together with rifampicin to mop up any unbound polymerase, the repression is overcome and RNA is made. It is clear therefore that repressor and polymerase do not compete for the same binding site; each can bind independently of the other and the repressor must act by blocking a step after polymerase is bound. Wild type promoter and operator sites are thus not the same nor do they overlap.

When, however, Pastan *et al.* repeated these experiments with *lac* operon DNA carrying a promoter mutation, which enhances the expression of the operon, they found that the addition of repressor after polymerase had bound to the DNA only partially inhibited RNA synthesis. By contrast, when repressor is bound before polymerase is added repression is complete and there is no transcription. These and other observations lead Pastan and his associates to conclude that the polymerase and repressor partially compete for a site on this mutated DNA. They suggest that either the mutation has, by deletion, brought the wild type promoter and operator sites closer together or, and more likely perhaps, the mutation has generated a new promoter which is closer to the wild type operator site. It is clear, therefore, that the same repressor molecule can bring about repression in more than one way.

Mechanisms of Sound Localization

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In this shortened version of his Char-nock Bradley Memorial Lecture delivered at the University of Edinburgh in November 1970, Dr Whitfield describes the mechanism whereby the differential intensity and time of arrival of a sound at the two ears allow the brain to determine the direction of arrival.

THEORIES of hearing have, from the earliest times, been overly concerned with frequency analysis, perhaps because of the classical interest in the relation between musical instruments and the physics of sound. Yet for survival in a hostile world, the role of the auditory system in identifying and locating the sounds made by friends and foes has undoubtedly been the more important one for most species. The early resonance ideas which concerned the cavities of the inner ear were indeed formulated more with the idea of providing an amplifying property than an analytic one, but as early as 1683 the Paris physician du Verney¹ proposed a resonance theory of frequency analysis, antedating Helmholtz by at least 150 years.

Theories of sound localization seem to have developed much later. Boring² has suggested that this was due to the philosophical climate of the early nineteenth century, which found the concept of auditory space difficult. Because of the ample evidence that the blind, for example, can find their way around using auditory cues, attempts to circumvent the difficulties induced by this philosophy were made by invoking tactile mechanisms as an explanation for sound localization.

By the middle of the century, experiment was beginning to take over, and with it the recognition that localization of sound depends on the differences between the stimulus conditions at the two ears³. An early attempt to control these differences was made by Alison⁴, who in 1858 described experiments with a "differential stethophone" which could be used to lead sound from a common source independently to the two ears. He showed that if the sound was louder in one ear than the other, then all the sound seemed to be in that ear, irrespective of the actual situation of the source. He interpreted his results as supporting the idea that localization depends on an intensity difference between the two ears.

The philosopher Alexander Bain, writing in 1855 (ref. 5), had to admit that the possession of two ears played some role in localization, but still insisted that this was very crude and that sound localization depended largely on "experience".

The work of the next hundred years was principally devoted to validating the importance of binaural phenomena and to demonstrating their accuracy; yet there is now reason to believe that past experience in the interpretation of auditory signals may well play some part in localization, though hardly to the extent which Bain supposed.

Rayleigh⁶ in 1877 investigated very thoroughly the localization of tones produced by tuning forks and firmly established

that intensity difference was a powerful factor in assessing the direction of a sound source. He realized, of course, that although these differences in intensity could be produced at high frequencies (short wavelength) by the shadowing effect of the head, it was difficult to account (in the absence of head movement) for our ability to localize low frequencies where shadowing is negligible. He thought that phase might play a part, and investigated this possibility by leading the sounds of two slowly beating tuning forks into the two ears through long tubes. He was unable to get any impression of movement of a sound image as the phases slowly changed and so rejected the idea. It was not until some thirty years later⁷ that he returned to the problem; he then reported that if the experiment were performed with the ends of the tubes separated from his ears by a short distance—instead of having the tubes actually in his ears—the impression of directional change was very definite.

In 1908 Mallock⁸ studied the apparent direction of the sound of rifle fire when he stood at different positions close to the line of fire. He found that not only was the apparent direction of the sound source not that of the rifle itself, but that the position of the source varied with the position of the observer along the line from gun to target. He concluded that what he was localizing was the shock wave of the bullet, and that this localization depended on the differential time of arrival of the crack at the two ears. This seems to have been the first study of the influence of a pure time difference in localization rather than a phase difference.

The First World War stimulated much experimentation in sound localization, and the development of electronic methods in acoustics in the 1920s and 1930s extended and elaborated the studies without adding anything substantially new. Study of the physiological mechanisms involved had to await a further stage in electronic development and these date from the late 1940s onwards. Many of the mechanisms are, indeed, still not understood.

Phylogenetic Development

In looking at what little is known, it is best perhaps to start with a look at the internal ear. It is now well known that a sound (say a single tone) sets up a travelling wave along the basilar membrane which passes from the basal to the apical end, reaching a maximum amplitude at some point and then rapidly dying out. The distance it travels before peaking and dying out depends on the frequency—the lower the frequency the further along the cochlea is this point. If a click with a sharp leading edge is used as a stimulus, then the higher frequencies are gradually filtered out as the wave travels along and only the lowest components arrive at the apex.

During phylogenetic development of the ear, the basilar membrane seems to have become progressively elongated. It is less than 1 mm long in reptiles, 5 to 10 mm long in birds and 20 to 30 mm long in mammals. Parallel with this there seems to have been an increase in the high frequency response. Reptiles (and fishes) respond only up to 1 or 2 kHz, birds reach 5 to 10 kHz, but among mammals the response of the guinea pig extends to about 40 kHz and that of the cat to 70 kHz. Man is, of course, a rather poor mammal with an upper limit of 20 kHz. This extension of the high frequency response is of interest because

of the importance of high frequencies for localization mechanisms.

The input data for the central nervous system—on the basis of which the source must be located—consist, of course, of impulses in the auditory nerves, and these impulses are in turn generated by mechanical movements of the hair cells induced by vibration of the cochlear partition of which they form a part. It seems that when the hairs of the hair cells are flexed in one direction there is an increased tendency for impulses to be initiated in the associated nerve, and a correspondingly reduced tendency for impulse initiation when they are flexed in the opposite direction. Certainly there is abundant evidence that nerve impulses tend to be discharged at a particular phase of a stimulating sinusoid⁹, and we can see at once that the time difference hypothesis and the phase difference hypothesis are—as far as the nerve is concerned—much the same thing. We have presumably to look for some central nervous mechanism which can assess the time disparity (Δt) between corresponding impulses in the two auditory nerves. It is not, however, quite as simple as that because an action potential lasts about 1 ms and the maximum delay between the arrival of a click at the two ears, even in humans, is about 700 μ s; in an animal such as the cat the delay is closer to 200 μ s. We can in fact deal with delays much less than this, such as those which occur when the sound is only a little off centre. Experiments performed by delivering clicks through headphones to the two ears separately indicate that delays of 40 to 50 μ s give an off-centre image. What is the mechanism? If we look at the rate of propagation of the travelling wave along the membrane we see that near the base it is travelling very rapidly¹⁰. To reach a point about 7 mm from the base takes only a few microseconds (this represents a position responding maximally to about 4 to 5 kHz). If we stimulate with a click containing high frequency components, then there is a barrage of impulses in the auditory nerve fibre array (several thousand fibres), which gives a statistical picture of the time of onset. For lower frequency pure tones we would expect localization to be worse, and this is, in fact, the case.

Comparing Information

How does the central nervous system deal with this time disparity information? Clearly, the information in the two auditory nerves has to be compared, and the first point, anatomically, at which this can happen is the superior olive, which receives connexions from both sides. The medial olivary nucleus has a remarkable structure; it consists¹¹ of bipolar cells with oppositely directed dendrites. The auditory input from one side makes contact with one set of these dendrites and that from the opposite side with the other set. The whole configuration is, of course, symmetrical with respect to the two sides of the brain stem. A number of workers, notably Hall¹², have recorded outputs from these cells while stimulating the two ears with paired clicks. The probability of a cell firing is found to be a function of the time disparity between the two signals. If the contralateral click is leading then the probability of a response is higher than if the ipsilateral click is leading; increasing the intensity of the clicks also raises the probability, of course. There are, however, two of these nuclei, one on each side, and we find that the ratio of the firing probabilities on the two sides is independent of the absolute intensity level. A difference of intensity between the two sides will affect this ratio, but this actually enhances the effect because the sound in the leading ear would normally be louder than that in the trailing ear if the stimuli came from a real single source.

The problem thus seems to be solved; some higher level of the nervous system has simply to assess the comparative outputs of the two medial olivary nuclei which vary with both time and intensity. When cats, for example, are deprived of both auditory cortices, they cannot localize sound (though they can respond to it—they are not deaf¹³); we note, too, that there are nerve cells in the cortex which respond only when the sound is on one side of the animal's head and not at all when

it is on the other¹⁴. If we postulate that these are the cells which are (directly or indirectly) "looking at" the differential olivary output, it seems superficially as if we may have identified the mechanism. Again, however, there are difficulties.

The relative development of the nuclei of the olivary complex varies considerably from species to species¹⁵. Some animals—the bat, the hedgehog, the dolphin—apparently have no medial olive, whereas the cat and the dog have the medial and lateral olivary nuclei about equally developed. In monkeys and man it is the medial olive which predominates, though in no species is the lateral nucleus entirely absent. It is interesting that these differences are reflected in nomenclature; in the cat it is the medial nucleus which anatomists have labelled the accessory nucleus, but in man it is the lateral nucleus which is so named. It is tempting to note the apparent correlation of these histological differences with head size; a medial olive is associated with a large head size, for which Δt would be large, and not with a small head size, for which Δt would be smaller. Before coming to any conclusions, however, we must recall that more "visual" species have larger medial olives than less visual ones.

Masterton, Jane and Diamond¹⁶ trained cats to distinguish between clicks presented by earphones to the right ear and to the left ear. They then presented the animals with paired clicks (right-left (RL) or left-right (LR)) in which one preceded the other by 500 μ s. As expected, they found that a normal cat treats RL just like a single click on the right and LR like a single click on the left. They then divided the trapezoid body so that the cross-connexions between the two accessory olives were severed. The animals could no longer solve the RL and LR problem. They could, however, still solve the L v R problem, so that relative intensity cues were evidently available by a different route. The experimenters further showed that partial lesions of the lateral lemniscus were capable of dissociating the two paths so that the L v R problem and the LR v RL problem were no longer treated as equivalent, that is both problems could be learned but there was no transfer.

Localization and the Pinna

We thus see that the time/intensity analysis of the accessory olive is not the sole mechanism of localization. Indeed when we come to look at the matter more carefully we see that time is a very ambiguous clue. There are in fact complete surfaces (hyperbolae of revolution) with the same time delays. Because of the irregular head shape and the existence of the pinnae, however, the relative frequency attenuation varies greatly with the orientation of the source. Thus the waveform at one ear is quite different from that at the other ear if the sound is complex (as it usually is) and this difference may be unique for each position. Batteau^{17,18} conducted many experiments on the role of the pinna in localization and showed how the received sound varies as the direction of the source is changed. Indeed this gives some indication of how we can locate sound even with one ear, though not very accurately. Such localization is, of course, not mechanical but depends on learning the patterns from past experience, just as we learn visual distance judgment from such things as atmospheric colour filtering. That there is some physical basis for the proposal is shown by the observation that artificial pinnae on microphones can be used to transmit the effect to a subject through earphones.

Now Rayleigh observed that when he had tubes in his ears leading in sounds he had no impression of direction, whereas when the ends of the tubes were an inch or so away he got the impression of direction very definitely. No doubt this is an example of the psychological effect of one clue cancelling the effect of another. If we perform on ourselves the experiment of delivering to our own ears through headphones clicks which are disparate in time—as in the experiments with the cat—we find that as the interval is altered, the sound certainly moves, but is lateralized rather than localized, that is to say it moves from side to side either inside your head or very close to it; it is not located out in space as is the case for a real

source. It seems that the frequency spectrum distortion provided by the head and pinnae is an important factor in this effect. But it may not be a complete explanation because Toole¹⁹ has recently shown that even real external sources in an anechoic chamber may in certain circumstances produce images inside the head.

There is, indeed, another factor in localization which we have not yet considered, and which may in normal circumstances contribute to the exteriorization of the source. So far we have been regarding a sound as two signals reaching the two ears at two points in time. This may be true in a large flat unpopulated open space, but in most circumstances these signals are accompanied by later versions reflected from surrounding objects. As sound travels roughly one foot in one millisecond, the sound of a voice reflected from a room wall may reach the listener five to ten milliseconds later, and there are many of these reflexions. Of course we do not hear them separately, and all the sound appears to come from the primary source. It is not that the reflexions are suppressed; they add to the total loudness, so that one must speak much more loudly in the open air than in a room. This phenomenon is known as the precedence effect²⁰, and is used commercially in sound reinforcing systems. The difficulty of situating a public address loudspeaker near the person talking is that it feeds back into the microphone and causes a howl. If, however, the speech fed to the loudspeakers is delayed so that their sound reaches the ear a few milliseconds later than the direct voice then the loudspeakers can be placed near the audience at the back of the hall and yet all the sound appears to come from the direction of the speaker's mouth.

With a single source on the left, the sound reaches the right ear about 0.5 ms later than the left ear. If, however, there is a reflector 3 feet away on the right, a second sound will arrive at the right ear 6 ms later and at the left ear 6.5 ms later. The auditory nerves will certainly respond to all four signals and indeed they can be traced in gross responses right through the auditory pathway, though the second response may be rather smaller than the first. Although, as we have seen, the second signal pair adds to the intensity of the whole and even to the intelligibility of speech, it is nevertheless the first signal pair which predominantly determines the apparent location.

Function of the Cortex

We have recently^{21,22} been investigating the role of the cortex in this phenomenon, as it seemed that the cortex played an important role in "out-thereness". First, we trained cats to go to a food box on the left when a loudspeaker behind that box was delivering a train of five tone pulses per second, and conversely to go to a food box on the right when a similar train of tone pulses came from a speaker on the right. In other words, they were trained to "go to" the sound. It is known from the experiments of Neff¹³ that a cat with no auditory cortex cannot do this but, provided either the right or the left auditory area is intact, the animal can perform the discrimination just like a normal animal.

If we intersperse among these real rights and lefts some compound rights and lefts made by having the right pulse precede the left by 5 ms or vice versa, normal cats will treat these exactly as if they were real rights or lefts—they "transfer" immediately without further training. This, of course, is just what we would expect from the precedence effect we have been discussing. If, however, we take out one auditory cortex a difference emerges. We removed the right auditory cortex from half of the animals and the left auditory cortex from the other half. After the operation both sets of animals made no mistakes about stimuli from the right or left: we did not expect them to. But there is a clear difference between the response of the two groups to the compound stimuli (RL or LR). The right lesion cats perform correctly on a compound right (RL) but fail on a compound left (LR), whereas the left lesion cats can do the compound left but fail on the compound right. In other words, the contralateral auditory cortex seems essential

for the correct interpretation of these compound signals. Some light on what actually occurs is given by other experiments in which uni-decorticate cats were trained to go left to a signal on the left, but to go right when signals were present on both right and left speakers without any fixed temporal relationship (to avoid the precedence effect the two trains were unsynchronized so that the left/right interval varied continuously). When these animals are exposed to compound signals the left lesion animals appear to be normal, they go left to a compound left (LR) and right to a compound right (RL) signal. But the right lesion cats go to the right whichever stimulus (LR or RL) occurs. With reversal training in which the animals are trained to go right to a single stimulus and left to the double stimulus it can be shown that the left lesion cats are not, in fact, normal, as they now break down and tend to go left to both RL and LR. These results suggest that it is the temporal order of the pair of stimuli which is lost when the cortex contralateral to the earlier member of the pair is damaged.

These observations may explain the difficulty which humans with one sided temporal lobe damage are reported to have in localizing sounds on the side contralateral to the damage, while having no difficulty with sounds on the ipsilateral side. Possibly the precedence effect breaks down in these circumstances, and reflexions cause confusion.

There are evidently comparatively simple and well understood mechanisms at the level of the brain stem which are capable of using the clues of differential intensity and differential time of arrival of a sound at the two ears to compute the direction from which it arrives, just as a range finder might. Some ambiguities in the result can obviously be resolved by moving the receivers (head movement) in the sound field, but only if the sound is repeated or continuous. In any real situation ambiguities arise not only because more than one position of the source can give rise to the same time differences but because of multiple reflexions. In the resolution of these ambiguities, the cortex seems to play an essential role, as it does in the assignment of position as well as direction to a source (what I have called "out-thereness"). It could well be asked whether "out-thereness" has any meaning except in so far as we are able to collate the auditory input at a cortical level with our motor experience of exploring the world around us.

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Effect of Earthquakes on Deep-sea Sediments

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It is suggested that sediments filling the oceanic trenches and transform faults suffer periodic liquefaction by earthquakes. Such a phenomenon could explain the presence of undeformed turbidite beds in highly tectonic zones and make them an ideal site for toxic waste disposal.

GEOLOGICAL and geophysical evidence from the mid-oceanic ridges generally supports the concepts of plate tectonics, but the interpretation of evidence from the areas where plates are colliding is much more obscure¹⁻⁹. One baffling problem is the almost complete lack of deformation in the sediments found in oceanic trenches^{10,11}, the "sinks" of plate theory, where one plate is under-thrusting another and being consumed into the mantle. Relative motion between colliding plates may be several centimetres per year⁸, and the boundary between them is marked by a steeply dipping earthquake zone, the Benioff zone, extending as much as 700 km into the mantle^{12,13}.

Several seismic reflexion profiles have now been made of the sediments filling oceanic trenches (Fig. 1), but there is no evidence for the accumulations of contorted sediments that were originally expected^{10,11}. One possibility is that crumpled sediments lie beneath the overlying undeformed fill, but cannot be detected because the latter is acoustically absorptive¹⁴. But beneath flat-lying turbidite beds Scholl *et al.* have identified oceanic pelagic sediment, very similar in thickness and acoustic properties to that exposed to seaward of the trench and beneath that a rather well defined basement. What evidence there is for chaotic reflexion hyperbolae at the base of the continental slope in active trenches¹⁵ can be explained by reflexions from sediments which have slumped down the continental slope¹⁶ or from harder rock features of

the slope itself. The two plates shown in Fig. 1 are moving together at about 6 cm yr⁻¹ (ref. 8) and, if the oceanic plate is carrying the trench sediments along on its back like a conveyor belt, a column of sediment approximately 1 km thick is constantly being pushed up against the continental slope. In a million years 60 km of such a column—more sediment than is visible in the whole of Fig. 1—must be disposed of. It is difficult to believe that any geological process can do this so efficiently that what is left can hardly be observed on a seismic reflexion record.

But it has always been assumed that the trench sediment is behaving as a plastic solid, displaying much the same mechanical properties as those of a sediment core resting on the laboratory bench. The static and dynamic physical properties of unconsolidated sediments are, however, seldom the same. In particular many unconsolidated sediments display the phenomenon of thixotropy, whereby a body changes from a semi-solid, jelly-like state ("gel") to a liquid state ("sol") on being shaken and reverts back to the jelly-like state on standing. Thixotropic behaviour by the sediments filling oceanic trenches under the influence of earthquake vibrations could account for their lack of deformation.

Vibrational Environment of Epicentral Zone

Such vibrations could originate in the Benioff seismic zone, which crops out along the inner margin of oceanic trenches^{12,13}. It is certain that much of the relative movement between colliding plates is accompanied by seismic activity. But fault movement can occur without earthquakes. For example, some sections of the San Andreas fault, separating the North Pacific and American plates, move with "fault creep", whereas others seem to be "locked" and strain is relieved by infrequent but severe earthquakes¹⁷. The locked stretches are thought to be the result of the curvature of the fault, that is, of increased forces holding the plates together. Because the Benioff zone involves underthrusting rather than strike-slip faulting, the forces normal to the zone must in general be greater than in the case of a transform fault. Thus the shallow faults of a

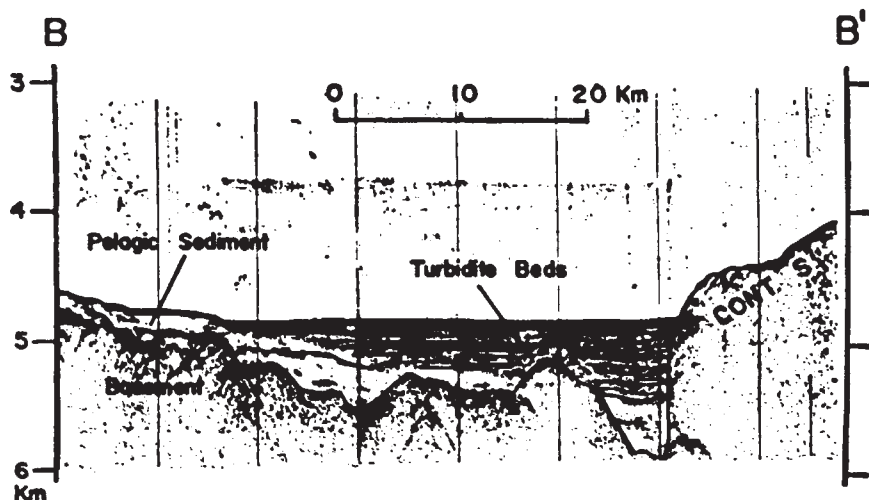


Fig. 1 Seismic reflexion profile taken by Scholl, van Huene and Ridlon¹¹ across the Peru-Chile trench, showing undeformed turbidite fill.

Benioff zone are probably of the locked variety. It seems plausible to conclude therefore that most, if not all, the relative movement between colliding plates is accompanied by earthquakes.

It is generally agreed that shallow earthquakes are produced by relative motion of the two sides of a fault surface¹⁸. Relative motion initiates at some point on the fault surface, then spreads outward on that surface at a finite velocity. Rupture velocities determined theoretically and experimentally give figures somewhat less than the shear velocity, or roughly half the compressional velocity, of the faulting material¹⁸⁻²². Thus P and S waves from the initial rupture will precede the active faulting everywhere except where it is initiated. At 5 km from the initial point, typical crustal rocks would experience approximately 1 s of vibration before the rupture starts. Near the source the vibration contains much high frequency energy (say, 2-100 Hz), most of which is attenuated by teleseismic distances. Moreover, the point at which rupturing starts is unlikely to be at the Earth's surface. Even where shallow earthquakes have resulted in surface faulting, it is usually thought that faulting started at some depth, possibly several kilometres²³. Thus the hard rock surface at the epicentre itself will usually experience at least a second of vibration before any faulting arrives.

During the Parkfield, California, earthquake of June 1966 an array of accelerographs was in operation across the part of the San Andreas fault which ruptured²²⁻²⁵. The earthquake had a Richter magnitude of 5.6 and the USCGS reported $m_b = 5.3$. The fault ruptured over a length of 30 km, the rupture initiating at a depth of about 5 km. Surface faulting was visible over a similar distance to the fault length inferred from Love Wave spectra, but offsets of the fault were only a few centimetres. A maximum acceleration of 0.5g was recorded 200 feet from the fault trace, falling to about 0.1g at a distance of 15 km^{24,25}. Much of the energy recorded in the accelerographs was at periods less than 1 s, and the shaking lasted for a few seconds. Although this earthquake is different in many respects from those occurring just beneath the ocean floor on the Benioff zone, these figures give a general idea of what to expect there from an earthquake of similar magnitude.

Housner²⁶ has shown that maximum ground accelerations decrease with distance from the fault, but that the rate of decrease is small over distances comparable with the dimensions of the fault. An earthquake such as the Parkfield one subjects a ground area of a few hundred square miles to maximum accelerations of more than 0.1g. A major earthquake with Richter magnitude 7.5 would subject an area of no less than 14,000 square miles to such accelerations, and an area of 2,000 square miles to maximum accelerations of more than 0.3g. The duration of strong shaking can exceed half a minute for major events.

Thixotropy of Unconsolidated Sediments

Boswell²⁷ has shown that, except for coarse clean sands and gravels, all unconsolidated sediments are thixotropic to some degree, and that thixotropy is augmented by an increase in the proportion of finely divided particles and of platy or acicular grains as compared with equidimensional particles. It is also usually increased by the presence of electrolytes such as those present in seawater as opposed to freshwater. Boswell found many sands to be thixotropic at porosities between 35% and 50%, muds and clays at porosities between 50% and 70%. The actual porosity range over which a given sediment exhibited thixotropy varied from a few per cent to 20%. Expressed in terms of the Atterberg limits of soil mechanics, all unconsolidated sediments are likely to be thixotropic near the liquid limit; the question is how far the thixotropic properties extend towards the plastic limit. Even at larger water contents than the liquid limit, thixotropy may be important in reducing the viscosity of sediment slurries.

Freundlich and Juliusburger have shown that thixotropy

can account for some quicksands²⁸. In particular, they demonstrated that a quicksand from Knott End, Fleetwood, was thixotropic due to the presence of only 2.1% of fine clay. Separation of the clay fraction suppressed the thixotropic behaviour, but it reappeared on remixing the clay with the residue. Sands neighbouring the quicksand contained only 0.3% clay and were not thixotropic.

In physical terms, thixotropy occurs when particles form a loosely packed "house-of-cards" structure²⁷⁻³⁰. Shearing stresses break the contacts between particles and thixotropy arises because time is required for the structure to reform. Particle shape is important because isometric particles tend to form close-packed structures whereas platy and acicular ones form open structures. Factors such as the concentration of electrolytes (affecting the electrostatic repulsive forces between particles) and the viscosity of the pore fluid are important because they control the time required for the structure to reform.

Physical Properties of Deep-sea Sediments

High porosity is perhaps the most striking characteristic of the unconsolidated sediments of the ocean floor^{31,32}. Porosities in the region of 70% are the rule and only rarely does the presence of an increased sand fraction reduce the porosity below 60%. The pelagic sediments of abyssal hills and the clayey silts and silty clays of abyssal plain turbidites all have porosities in the region of 80%. Furthermore, porosity decreases slowly with depth. At the Preliminary Mohole site it fell only 5% over 168 m (ref. 33). The sound velocity gradient in unconsolidated sediments and the well established relationships between sound velocity and sediment porosity provide an indirect means of inferring porosity at depth. One can conclude that even at depths of 1 km below the ocean floor, the porosity of unconsolidated silt-clay sediments is probably in the region of 50%. The fact that high porosity extends to depth implies that a house-of-cards type of structure does also, with only gradual orientation of the sediment particles towards the structure of laminated siltstone or shale.

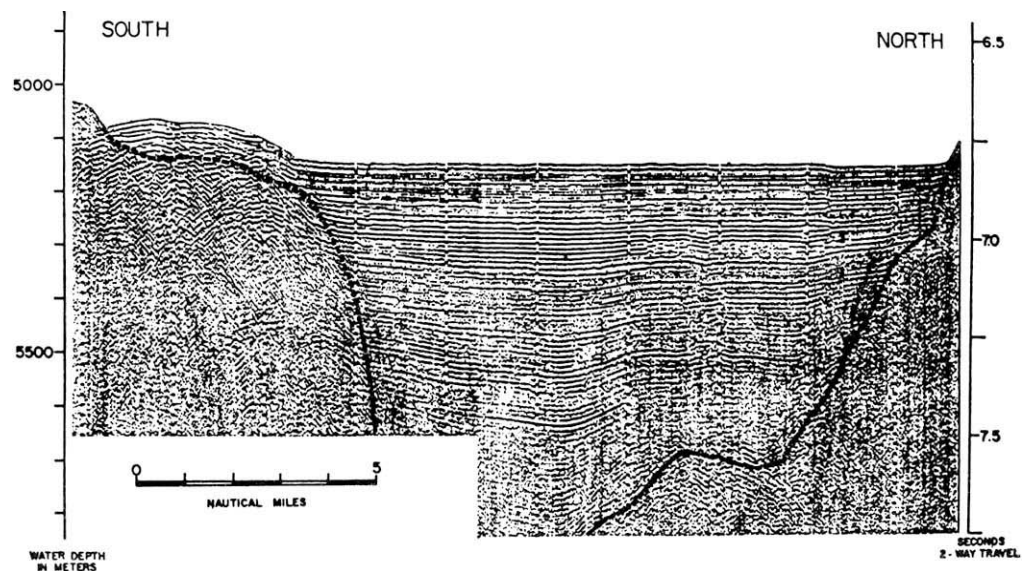
Porosity alone, however, is not a reliable guide to either the strength of an unconsolidated sediment or its closeness to the liquid limit. In general, the shear strength of deep-sea sediments is low, but the strength of sediments of similar porosity may vary by a factor of a hundred or more^{32,34}. Hamilton has pointed out that both very high and very low rates of accumulation can lead to sediments with little decrease of porosity with depth³³. When the rate of accumulation is very high, the sediment layer is thick and very weak, but has not had time to consolidate under its own overburden pressure³⁵. When the rate of accumulation is very low (say, <1 cm per 1,000 yr), the contacts between sediment particles are firmly cemented before the overburden pressure becomes appreciable. The house-of-cards structure is thus transformed into a welded three-dimensional network capable of supporting a considerable overburden and giving the sediment appreciable strength. This is the explanation given for the surprisingly high strength of the sediments from the Preliminary Mohole^{33,36}, and is probably generally true of deep-sea pelagic sediments.

Thus the properties of many sediments on the ocean floor are such that, given the right vibrational environment, thixotropy is likely to be an important phenomenon. Rapidly deposited turbidite beds are more likely to display thixotropy than slowly deposited pelagic clays. No quantitative information is available on the level of vibration needed to induce thixotropy, but there is a great deal of evidence that the strong motion of earthquakes is sufficient to disturb the inter-particle relationships of unconsolidated sediments.

Effects of Earthquake Strong Motion

Liquefaction of saturated, cohesionless sands and silts has been reported in several earthquakes³⁷, but the mechanism of

Fig. 2 Seismic reflexion profile taken by Van Andel, Corliss and Bowen⁴⁵ across the Vema Fracture valley, showing undeformed horizontally bedded sediments.



liquefaction when this occurs on land is generally thought to be moving pore water rather than thixotropy. Earthquake vibrations tend to shake cohesionless sands and silts into a more close-packed structure and thus increase the pore water pressure. The pore water flows upwards towards the surface and brings about liquefaction. The much smaller grain size of deep-sea sediment makes this mechanism less likely than thixotropy on the ocean floor, but the fact that it has been observed to have taken place on land demonstrates that the strong motion of earthquakes does disrupt the inter-particle relationships of unconsolidated sediments.

It is now well established that earthquakes provide a common triggering mechanism for submarine turbidity currents³⁸. The classic example of this occurred off the Grand Banks of Newfoundland in 1929, when a magnitude 7.2 (Richter) earthquake³⁹ set a turbidity current in motion down the southern slope of the Banks. The velocity of the current has been inferred from the sequence of telegraph cable breaks⁴⁰ and its extent has been demonstrated by coring⁴¹. Sediment across 100–200 km of the continental slope was set in motion by this earthquake. The important point is that the level of earthquake vibration was sufficient to disturb the inter-particle relationships of the unconsolidated sediment throughout the epicentral area.

Liquefaction of Sediments in Oceanic Trenches

The various threads of the argument can now be drawn together to explain the absence of deformation observed in the sediments of oceanic trenches.

Throughout their existence, the turbidite beds of oceanic trenches will have experienced an unusual environment for marine sediments. Disturbed by the strong vibrations of frequent earthquakes, their inter-particle relationships will not have been static. Liquefaction, probably brought about by the phenomenon of thixotropy, will have periodically broken the contacts between particles and prevented their becoming permanently cemented.

In contrast, the pelagic sediments, approaching the trench on the oceanic plate, will have already acquired considerable strength as a result of inter-particle cementing before they reach the earthquake zone. The vibrational environment could therefore liquefy the turbidite beds but is unlikely to have such a drastic effect on the bulk of the pelagic sediments. Furthermore, since the initial ruptures of earthquakes generally lie at least a few kilometres below the base of the sediments, liquefaction of sedimentary formations could already occur before the appearance of faulting in the basement surface.

The brief and intermittent earthquake movements cannot impose shear stresses on the liquefied body of sediment and the latter will be effectively decoupled from the oceanic conveyor, just as the sea itself is. The situation is comparable with that of a bowl of water given a sharp tilt. The bowl moves, but the water level remains the same. Similarly the liquefied sedimentary pond in the trench retains its level and horizontal stratification as the oceanic plate slips by beneath. Moreover, although the cumulative movement of the oceanic plate, towards and underneath the continental plate, is quite rapid in geological terms, the actual shape of the sedimentary trap of the trench probably retains a rough dynamic equilibrium. Over a long period of time, several tens of millions of years, the relative velocities between plates will change and trenches come and go. But over a shorter time term, up to a few tens of millions of years, the relative velocities between the plates are probably quite constant. The general position of trenches would not vary although local adjustments of depth might occur. This might give rise to liquefied sediment redistributing itself along the trench. Such flows would not alter the character of the trench fill, but rather would tend to maintain its horizontal stratification and low strength.

Liquefaction of Sediments along Transform Faults

Besides the island arc/trench systems, the other prominent submarine earthquake zone follows the axis of the mid-oceanic ridges⁴². In general, of course, the crest of the mid-oceanic ridge is such a new feature that no appreciable sedimentary cover exists⁴³, but some fracture zones form troughs deep and extensive enough to trap turbidite sediments originating well away from the ridge itself. The Vema Fracture zone^{44–47} in the equatorial Atlantic offsets the mid-Atlantic ridge by more than 300 km, is the locus of earthquake epicentres, and is filled by more than 1 km of horizontally stratified sediments, predominantly turbidites from the Demerara abyssal plain (Fig. 2). Interpretation of magnetic anomalies indicates that the north and south walls of the Vema transform fault are moving past each other at 2.8 cm yr⁻¹ or, expressed differently, that the basement on the south side of the profile shown in Fig. 2 is about 20 million years older than that on the north side⁷. Nevertheless, the sedimentary fill observed is essentially undeformed.

Van Andel *et al.* originally interpreted the lack of deformation in the sediments of the Vema Fracture to indicate a long period of tectonic tranquillity, but were still convinced of the tectonic nature of the walls of the fracture⁴⁵. A recent JOIDES drill hole coinciding with the profile of Fig. 2 has established an

exceptionally high sedimentation rate in the Vema Fracture Valley, of the order of 1 m every 10^3 yr⁴⁷. Even this rate, however, is but a small fraction of the rate at which the north and south walls of the transform fault are moving past each other and rapid sedimentation alone cannot account for the lack of deformation. Again the answer may lie in the effect of earthquakes on the physical properties of the sediment itself. The sediments filling the Vema Fracture have been shown to be rapidly deposited turbidites and consequently liable to thixotropy. Provided that movement on the Vema transform fault is largely seismic, the sedimentary fill in the fracture will be liquefied during movement of the walls and consequently will escape deformation and retain its horizontal stratification. It is quite reasonable to suppose that the largest earthquakes on the transform fault produce faulting throughout its 300 km length. In such an event the whole sedimentary fill of the transform part of the fracture and for many kilometres on either side would be liquefied.

Conclusions and Consequences

On a geological timescale liquefaction will be a frequent but transient occurrence, at any particular place averaging the order of seconds per year. Several months may go by without liquefaction, and at much longer intervals very large earthquakes will induce it for half a minute or more—a period comparable with their duration of strong shaking. Liquefaction implies a reduction in the viscosity of the sediment from that of a plastic solid (say, 10^4 poise) to that of a thick soupy liquid (say, 10 poise) and the loss of the plastic solid's yield strength.

Bostrom and Sherif have recently suggested that the tectonic sinks of the Earth's surface be used for waste disposal⁴⁸. The occurrence of liquefaction in the turbidite beds of oceanic trenches would make this suggestion much more plausible. Furthermore, thickly sedimented transform fault valleys could be put to the same use. Waste material packaged to a density greater than that of the sediments would sink through the sediment whenever it liquefied. It is worth making an estimate of the rate at which a solid body would sink through the liquefied sediment.

Consider a sphere of diameter $d=100$ cm and density $\rho_s=3.0$ g cm⁻³ falling at a velocity U through a liquefied sediment of density $\rho_L=1.5$ g cm⁻³ and viscosity $\nu=10$ poise. The Reynolds number in such a case will be of the order of 10^3 so that the drag force the body experiences is independent of the viscosity of the liquid and is given by

$$F = \frac{1}{2} C_D U^2 S \rho_L$$

where S is the cross sectional area perpendicular to the direction of the motion and C_D is a dimensionless number called the drag coefficient. For a sphere $C_D \approx 0.4$ for Reynolds numbers in the range 10^3 to 10^5 (ref. 49). The terminal velocity of the sphere is given by

$$\frac{4}{3} \pi \left(\frac{d}{2}\right)^3 (\rho_s - \rho_L)g = \frac{1}{2} C_D U^2 \pi \left(\frac{d}{2}\right)^2 \rho_L$$

and therefore

$$U^2 = \frac{4}{3} \left(\frac{\rho_s - \rho_L}{\rho_L}\right) \frac{gd}{C_D}$$

Thus, with the values given, $U \approx 6$ m s⁻¹. Because the motion at these high Reynolds numbers is independent of the viscosity of the fluid, the velocity with which the sphere sinks through the sediment is only a factor of about $\sqrt{2}$ less than that at which it sinks through the sea. The duration of liquefaction has been tentatively estimated at s yr⁻¹, so that the average rate of sinking through the sediment of a body such as the sphere considered above might be about 20 m yr⁻¹. This rate far exceeds geological sedimentation rates and could be verified experimentally with simple apparatus in a few years.

That waste material dropped on the turbidite beds filling oceanic trenches and transform fault valleys would sink rapidly through the sediment is an attractive concept. Burial to a depth of a kilometre or more would occur in the human lifespan. As a site for the disposal of highly toxic chemical or radioactive wastes, the sedimented trenches and transform faults appear to be ideal. Such wastes have been dumped at sea for several years with remarkably little thought given to the consequences. Yet a major problem in the disposal of highly concentrated radioactive waste is the difficulty of ensuring that the package will last at least a few half-lives of the nuclides it contains. If natural geological processes exist which would allow such packages to bury themselves to a depth of a kilometre or so in sediment and this beneath seawater 5 km deep, such difficulties do not arise. A guaranteed lifetime of 50 yr for the package would suffice. And if leakage of the toxic waste then ensued, its effect even on the benthic environment would probably be undetectable.

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Murine Leukaemia Virus Group-specific Antigen in the C-Type Virus-containing Human Cell Line, ESP-1

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The C-type virus particle in ESP-1 cells seems to be a murine virus.

IDENTIFICATION and classification of non-cytopathic viruses isolated from human tissues or human cell cultures require a combination of morphological, biological, biochemical and immunological tests. Because of their characteristic budding appearance at the plasma membrane, C-type leukaemia-sarcoma viruses are usually identified by morphological criteria¹. Further classification as to species of origin can be accomplished immunologically. Mouse, hamster, cat and rat C-type viruses each contain an internal virion protein, the group-specific (gs) protein² which carries species-specific antigenic determinants³⁻⁵. Thus, C-type viruses of these species can be identified specifically by a number of different immunological tests. C-type particles have been described in a human cell line, ESP-1, derived from a patient with lymphoma, and the results indicated that this putative human virus has none of the known gs antigens⁶. We describe here immunological studies showing that the ESP-1 cells contain an antigenic determinant identical to the species-specific antigenic determinant of murine C-type viruses.

The ESP-1 cell line was obtained at the forty-first passage level through the courtesy of Drs Elizabeth Priori and Leon Dmochowski (M. D. Anderson Hospital and Tumor Institute, Houston, Texas). The original cultures were received as monolayer cultures and maintained in two different laboratories throughout these studies. Extreme care was taken to avoid contact with other C-type virus-infected cell cultures: this included use of isolated work space and maintenance of flasks in incubators containing only ESP-1 cultures. Media consisted of McCoy's plus 20% foetal calf serum or RPMI 1640 plus 10% heat inactivated foetal calf serum. Although the ESP-1 cell line is contaminated with mycoplasma, no antibiotics other than penicillin and streptomycin were used.

To determine the immunological relationship of the C-type virus in ESP-1 cells with the known mammalian C-type viruses, complement-fixation (CF), radioimmunoprecipitin inhibition (RIPI), and gel diffusion studies were performed. The following reagents gave consistent positive reactions in CF tests with 10–20% v/v ESP-1 cell homogenates: eight serum pools from rats bearing murine sarcoma virus (MSV) induced tumours which were selected for reactivity with the mouse gs antigen⁷ and serum from two rabbits and three guinea-pigs immunized with the purified⁸ gs antigen of mouse C-type viruses (MuLV). In contrast, no CF reactivity was obtained with guinea-pig antiserum prepared against purified feline (FeLV)⁴, rat (RaLV), or hamster (HaLV)⁹ gs antigens. As Table 1 shows, concen-

Table 1 Reactivity of ESP-1 with Representative Antisera directed against Mammalian C-Type Viruses as detected by CF and RIPI

Sera *	Test	Antigen †	Antigen titre or range ‡
Rats with MSV-induced tumours	CF	MuLV	16– 64
		ESP-1	2– 8
Guinea-pig anti-murine gs	CF	MuLV	16–128
		ESP-1	2– 8
	RIPI	MuLV	250
Guinea-pig anti-feline gs	CF	FeLV	64–128
		ESP-1	< 1
Guinea-pig anti-hamster gs	CF	HaLV	8– 16
		ESP-1	< 1

* Sera were tested at 4 antibody units as defined by the reaction with purified gs antigens.

† Antigens were 10–20% (v/v) cell homogenates prepared from virus-releasing cell cultures. In all cases uninfected cell controls prepared in an identical manner failed to react with these sera.

‡ Expressed as the reciprocal of the highest antigen dilution showing 3+ or greater fixation of C' (CF), or 50% or greater inhibition in the RIPI test.

trated ESP-1 cell homogenates had antigen titres eight to ten times smaller than routinely obtained with the homologous murine systems (murine leukaemia virus grown in rodent cells). The regularity with which positive reactions were obtained at different cell passage levels, with different sources of anti-murine gs sera and in different laboratories, strongly suggested cross-reactivity in ESP-1 cells with MuLV gs antigens. Equally important in indicating specificity for MuLV was the absence of reactivity in CF tests with ESP-1 cell extracts and antisera which detect feline and hamster gs antigens.

Further evidence that the ESP-1 antigen was cross-reactive with MuLV gs antigen was obtained by RIP inhibition (RIPI) tests. The double antibody precipitation method was used¹⁰ with free and antibody-bound antigen being separated by centrifugation through sucrose gradients. This method permits a clear separation of free antigen (molecular weight ~25,000) which remains in the upper 10% of the gradient and antibody-bound antigen which is sedimented. MuLV gs antigen was labelled with I^{125} (5×10^5 c.p.m./ μ g protein) by the chloramine-T method¹¹ and only used after a final purification through SDS-polyacrylamide gels⁹. The precipitation tests were made in conditions in which a specific anti-MuLV gs rabbit serum was diluted to give 50% precipitation (serum dilution 1 : 5,000) of approximately 0.01 μ g of labelled antigen. Only homogenates of cells infected with known strains of MuLV and the ESP-1 cell showed any measurable inhibition, while cells infected with hamster or feline C-type viruses and uninfected human, hamster and mouse cells gave negative results. ESP-1 antigen titres by RIPI were consistently twenty times smaller than comparably prepared MuLV antigens from murine cell cultures.

To establish fully the relationship of the immunological determinants of the antigen in ESP-1 cells with murine C-type virus gs antigen determinants, it was necessary to show precipitin lines of identity in immunodiffusion tests, using sera specific for the murine gs antigen. Because of the relatively low antigen concentration, 50–100% (v/v) ESP-1 cell concentrates or highly concentrated supernatant fluids were used. Figs. 1A and B show two examples of precipitin lines of identity between highly purified murine gs antigen and ESP-1 concentrates using different sets of reagents prepared and tested in different laboratories. Both rabbit and guinea-pig anti-MuLV gs sera have given lines of identity using several ESP-1 preparations and purified MuLV gs antigen. Although the identity reactions were most clearly seen when ESP-1 preparations were placed between positive MuLV antigens, definite identity reactions were also seen if a positive control was placed in only one adjacent well. The specificity of the reaction shown in Fig. 1B was indicated by the lack of curvature of the precipitin lines when control antigens were placed in well 6. The diagnostic antisera did not react with FeLV, RaLV, or HaLV antigens, nor did they react with uninfected human cells grown in the same media. In parallel tests using antibody specific for FeLV, RaLV and HaLV gs antigens, no reactions were obtained with any of the ESP-1 preparations. These data show that the ESP-1 cells possess an antigen with determinants identical to those detected on the group-specific antigen of the murine C-type viruses.

Mouse C-type leukaemia-sarcoma viruses have previously been shown to grow in human cells^{12–15}. Further, mouse and cat C-type viruses which have been grown in human cells for long periods in culture retain their original species-specific gs antigen reactivity¹⁶. These facts, together with the data presented above, suggest that the C-type virus particle in ESP-1 cells is a murine virus. Immunological identity of mouse and human C-type virus gs antigens, although remote, remains an alternative explanation of the present data.

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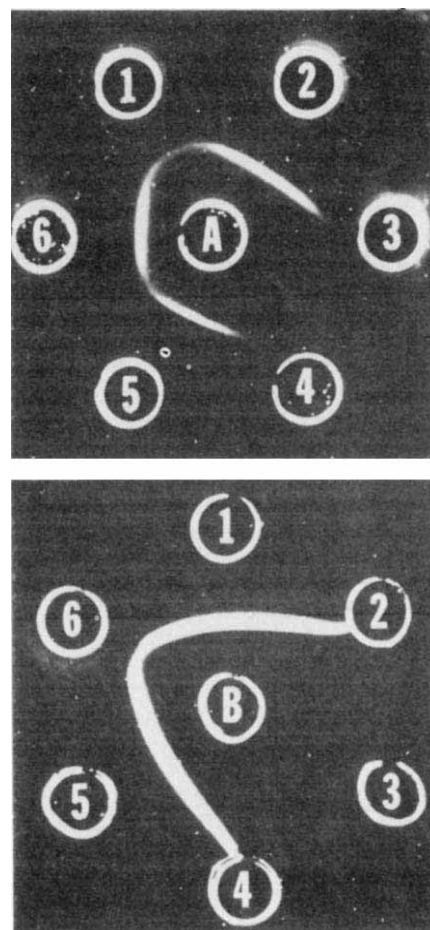


Fig. 1 Immunological identity of antigenic determinants in ESP-1 cells with MuLV gs antigen. Wells contained: Laboratory A, 1, human ESP-1 cells, 50% v/v; 2, MuLV-infected mouse cells (Kirsten strain); 3, human diploid fibroblast cells, 50% v/v; 4, FeLV (concentrated, ether disrupted); 5, MuLV-infected mouse cells (Rauscher strain); 6, purified MuLV gs; A, Rabbit 1 anti-MuLV gs. Laboratory B, 1, purified MuLV gs; 2, HaLV-infected hamster cells; 3, FeLV-infected cat cells; 4, RaLV-infected rat cells; 5, MuLV-infected rat cells; 6, ESP-1 concentrated supernatant fluid; B, rabbit 2 anti-MuLV gs.

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Virus-like Agents from Patients with Hodgkin's Disease

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Cultured cells from patients with Hodgkin's disease underwent changes which seemed to be accompanied by the emergence of an agent of the herpes group.

THE aetiology of Hodgkin's disease is unknown. But the age incidence of the condition¹, the fact that an individual who has a close relative with this disease is three times more likely to contract it², and recent evidence of possible case-to-case transmission³, all suggest that infective agents may be involved, and that these may be viruses, although other kinds of agents have been considered in the past⁴.

Two difficulties have beset all attempts to implicate particular viruses as a cause of human tumours. Most such attempts involve tissue culture studies which must begin with biopsy specimens containing more than one cell type; thus, when cell lines have been successfully established in culture, it is uncertain whether these are indeed the malignant cells. Second, tumour cells seem to be especially favourable for the multiplication of viruses, and frequently harbour passengers which are not involved in the induction of malignancy. Hodgkin's disease presents an additional difficulty. Long recognized as a mixed neoplastic and inflammatory condition, the actual malignant cells have not been positively identified even in pathological specimens, although the classical Reed-Sternberg cells may be one such⁵. In this article we report that: (a) we have established from patients with Hodgkin's disease a number of long term cell cultures which we suspect may contain the malignant elements; (b) these cells, in culture, seem to release an RNA-containing agent into the supernatant medium; (c) certain of these cell cultures regularly undergo "transformation" in culture, with the appearance of a large number of rounded, free floating cells, certain populations of which become capable of independent growth in suspension; (d) such free floating cells release into the culture medium, in addition to the RNA agent already mentioned, a second agent containing DNA; (e) the latter has been tentatively identified as a member of the herpes group, though its reactions are typical of neither herpes simplex nor Epstein-Barr virus [EBV, HLV]. The relationship of these agents to either the aetiology

or the pathological development of Hodgkin's disease is by no means clear.

Establishment of Cell Cultures

Early attempts to cultivate lymph node material from Hodgkin's disease using classical coverslip or plasma-clot methods⁷ gave cultures of predominantly mixed cell type, which died out by the end of the second week or earlier. Subsequently, a long term, free floating cell line, consisting of several types of cell, was obtained by Ito *et al.*⁸, and a similar one by Pontén⁹. In the latter case the free floating cells had arisen by "lymphoblastic transformation"¹⁰ of an apparently random growing layer of fibroblast-like cells; the free floating cells could not be cultivated in the absence of a feeder layer.

The culture method we have used depends on the fact that biopsies of Hodgkin's disease lymph nodes contain many differentiated cells such as lymphocytes and macrophages, which may be capable of at most a few divisions in culture. It was assumed that the eventual death and disruption of such cells might release products inimical to the growth of undifferentiated cells potentially capable of giving rise to cell lines. This was consistent with our failure, as of others, to initiate cultures with five trypsinized lymph node biopsy specimens, trypsinization being notoriously destructive of cells of the lymphoid series. The method finally adopted was a modified Trowell-type organ culture¹¹. Fragments of lymph node 0.5–1 mm in diameter were placed directly on stainless steel grids, two to four pieces per grid in organ culture dishes¹²; the medium was Eagle's MEM + 15% foetal calf and 10% pretested human serum. The pieces remained on the grids for at least 3–4 weeks, during which time they continued to shed cells onto the surface of the dish below. As soon as the latter became covered by a confluent layer of cells (mostly round cells plus fibroblasts), the grid was transferred to a new dish, such transfers being repeated at intervals until cells were no longer deposited. The pieces of node were then transferred without trypsinization to 60 mm Petri dishes plus 5 ml. of medium; the shed cells could rarely be transferred more than a few times, and were discarded.

In all cases cells now grew out from the node fragments which were very large, pleomorphic and thinly spread, with a markedly reticular cytoplasm, many lysosomes and sometimes eosinophilic inclusions and will henceforward be termed

attached cells. They were mostly mononucleated, though sometimes binucleated, with prominent nucleoli varying in number from one to four (Fig. 1). They grew individually and showed neither a fibroblastic nor an epithelial pattern of growth. When confluence was achieved, the cells in the layer were randomly oriented and could now be passaged following trypsinization. They retained their distinctive morphology for at least seven or eight passages (7–8 weeks), and cultures stored at -78°C at earlier passage levels gave rise to attached cells when revived.

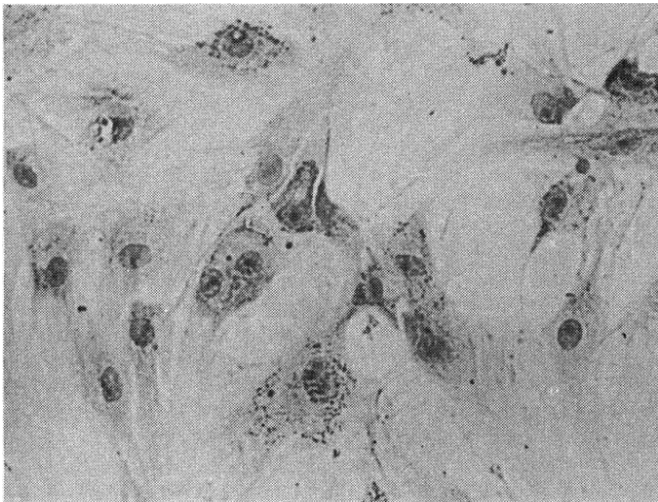


Fig. 1 Photomicrograph of cells from attached cell line (SK-HN-5b). Large, randomly arranged cells with one or two nuclei and prominent nucleoli. Reticulated cytoplasm with lysosomes ($\times 192$).

The chromosomes of the attached cell lines derived from five patients of both sexes had a modal number of forty-six, the diploid number for man. In one of these five (a male) about two-thirds of the 104 metaphases counted were hypo-

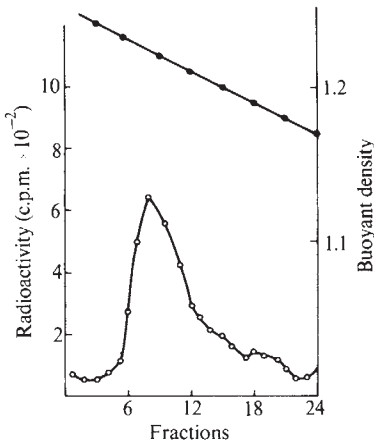


Fig. 2 Equilibrium density centrifugation in 20–58% sucrose of pelleted ^3H -uridine-labelled material from supernatant fluid of attached cell line SK-HN-CH.N, labelled as described in the text (16 h, 27,000 r.p.m., SW.27 rotor, 5°C). ●—●, Buoyant density; ○—○, radioactivity (c.p.m.).

diploid (forty-five) owing to the absence of the *Y* chromosome. Thirty-eight of fifty fully analysed karyotypes were normal, and among the twelve remaining aberrant karyotypes there were no consistent structural or numerical abnormalities. Thin sections of such cells, examined with the electron microscope, showed nuclei with dispersed euchromatin and large nucleoli. Occasional perichromatin granules were noted, very close to a thin rim of marginated heterochromatin. The cytoplasm was extremely rich in organelles of all types, an endoplasmic region being frequently distinguishable from peripheral ectoplasm. There were very many dilated rough endoplasmic reticulum cisternae containing proteinaceous material of moderate electron density, and several foci of Golgi structures. The ectoplasm showed many fewer organelles, occasional mitochondria and lysosomes being scattered among bundles of microfilaments. Structures that could be unequivocally identified as virus particles have not been seen so far.

Table 1 Attempts to Establish Cell Strains from Lymph Nodes, Skin, or Tumour of Patients with Hodgkin's Disease

Subject	Cultured organ	Age at biopsy (yr)	Sex	Time on grid (weeks)	Tissue in culture (weeks)	No. of cell generations	Cell strain
Hodgkin's patient 1	Lymph node	14	♂	7	27	34	SK-HN-1
Hodgkin's patient 2	Lymph node	8	♀	5	36	48	SK-HN-CH.H
	Lung tumour	9	♀	6	34	92	SK-HT-CH.H
	Skin	9	♀	2	19	70	SK-HS-CH.H
Hodgkin's patient 3	Lymph node	22	♀	3	17	40	SK-HN-3
	Spleen	22	♀	5	30	30	SK-HSp-3
Hodgkin's patient 4	Lymph	23	♂	4	17	28	SK-HN-4
Hodgkin's patient 5	Lymph	52	♂	6	10	18	SK-HN-5a
					7	12	SK-HN-5b
				16	24	180	SK-HNF-5a
					20	150	SK-HNF-5b
	Skin	52	♂	6	16	30	SK-HS-5
Hodgkin's patient 6	Lymph	15	♂	6	16	26	SK-HN-6
Hodgkin's patient 7	Lymph	23	♂	5	17	32	SK-HN-7
Hodgkin's patient 8	Lymph	14	♂	7	17	14	SK-HN-8

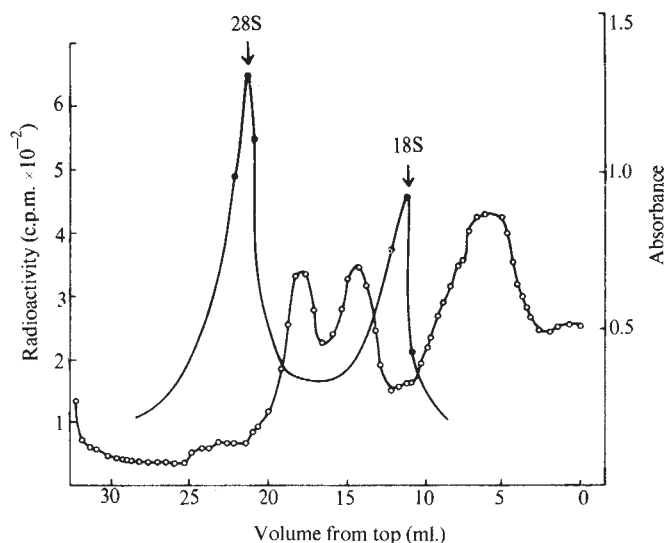


Fig. 3 Velocity sedimentation in sucrose (15–30%) of ^3H -RNA from the isopycnic labelled band at 1.20–1.22 liberated with SDS after dialysis to remove sucrose. 18S and 28S RNA markers from whole rat liver ribosomes (14 h, 25,000 r.p.m., SW.27 rotor, 10° C). ●—●, Abundance at 260 nm; ○—○, radioactivity (c.p.m.).

Using the above technique, it was possible to derive attached cell cultures of the same type from ten patients. In the case of eight of them (Table 1) they have now been carried for up to forty-eight cell generations. The other two were stored frozen at an early passage level; the cells, however, had a similar morphology, and none had reached "crisis"¹³ before storage. In contrast, cells cultured from two normal lymph nodes went into "crisis" and failed to become established (Table 2). In two Hodgkin's cases (patients 2 and 5, Table 1) skin biopsies also gave rise to cell strains (SK-HS-CH.H, SK-HS-5), whose cells were not typical skin fibroblasts but resembled the attached cells obtained from the lymph nodes of the same individuals. One of these patients had clinical

mother of patient 2 (Table 2), which did give rise to a continuously propagable line of fibroblasts. Patient 2, however, was homozygous for the Chediak-Higashi syndrome¹⁴, and her mother by definition a heterozygote; it is not known what influence this rare genetic anomaly may have on the ability of fibroblasts to propagate indefinitely in culture.

Release of Agent containing RNA

To establish whether the attached cell lines could be releasing anything resembling a virus, a search was made in the tissue culture media for material excreted by the cells which might have biophysical properties like those of viruses¹⁵. ^3H -uridine was incorporated in the medium at a concentration of 1 $\mu\text{Ci}/\text{ml}$. Cells were labelled in log phase, 24 h after splitting semiconfluent cultures, and allowed to grow in labelled medium for 4 days. The medium was then removed (minimum volume per experiment = 300 ml.), clarified by centrifugation at 6,000 r.p.m. for 30 min at 5° C. It was then filtered through a 22 nm 'Millipore' membrane filter and pelleted at 15,000 r.p.m. for 2 h at 5° C in an SW.27 rotor. The supernatant was discarded and the pellet resuspended in three drops of Tris-EDTA buffer (0.01 M Tris, 0.001 M Tris, 0.0001 M EDTA, 0.1 M NaCl, pH 7.2), and layered over a 15–58% w/w sucrose gradient. The gradient was then centrifuged to equilibrium, and the gradient fractions collected and assayed for radioactivity. A typical result is shown in Fig. 2. A marked peak of radioactivity banded at a sucrose concentration corresponding to a density of 1.20–1.22. Two of our attached cell lines have been studied in this manner, and have consistently given the same result (six experiments).

Five other human and one hamster cell line were similarly labelled and treated to serve as controls. Cells from (1) a neuroblastoma (SK-N-SH), (2) a breast carcinoma (SKBR-3), (3) a continuous "floating" cell line (SK-LN) from a normal lymph node, (4) strain WI-38 embryonic lung fibroblasts, (5) the FL continuous line of amnion cells, all failed to show any uridine-labelled peak when similarly treated. (6) B34 hamster cells, a suspended cell culture line that continuously releases hamster sarcoma virus¹⁶, served as a positive control; when labelled in the same way it gave a peak sedimenting at a density of 1.14–1.15. After labelling the attached cell lines in a similar manner with ^3H -thymidine, no peak was found.

Table 2 Attempts to Establish Cell Strains from Lymph Nodes and Skin of Individuals without Hodgkin's Disease

Subject	Cultured organ	Sex	Time on grid (weeks)	Tissue in culture (weeks)	No. of cell generations
Control patient 1	Lymph	♂	4	8*	14
Control patient 2	Lymph	♀	5	12*	12
Control mother of Hodgkin's patient 2	Skin	♀	2	20	70
Control patient 3 (normal)	Skin	♀	2	20*	28

* Cell strain went through "crisis" at this time and expired.

evidence of skin infiltration by tumour cells (patient 5). That the attached cells cultured by our technique may indeed be malignant was also suggested by preliminary heterotransplantation experiments in which 10^6 cells of the culture line obtained from patient 6 (SK-HN-6, Table 1) were inoculated into the cheek pouches of cortisonized weanling Syrian hamsters. Six out of twelve such inoculations gave rise to small tumours which began to regress after 1–2 weeks; 10^6 cells inoculated into the dorsal region of each of six newborn Syrian hamsters did not give rise to tumours. Cells from the skin of a normal individual gave typical fibroblast cultures which went through crisis and died out. An exception was the skin from the

It was then necessary to establish that the ^3H label in the 1.20–1.22 sucrose density peak was in fact in RNA, and that, if so, the RNA was not of cellular (for example, ribosomal) origin. Fig. 3 shows the result of a sedimenting ^3H -labelled RNA extracted from the 1.20–1.22 band on a sucrose velocity gradient at the same time as ribosomal markers. None of the three bands of labelled macromolecular RNA coincided with the 28S and 18S bands of the marker. The RNA is thus clearly not derived from ribosomes. It is, however, undoubtedly RNA, since treatment of the material with RNAase eliminated the peaks, all radioactivity now sedimenting at the top of the gradient.

There remained a trivial explanation that the ^3H -uridine peak was due to mycoplasma contamination; this, however, was ruled out by means of experiments to be described later.

The breadth of the labelled RNA peak (Fig. 2), however, indicates that the isopycnic band is still markedly heterogeneous. This is confirmed by electron microscopy of the material in the band. A range of particulate material is present, among which it has not yet been possible to identify any virions of characteristic morphology.

"Blastoid Transformation"

Cultures from the lymph nodes of two of the patients listed in Table 1 (patients 5 and 2) eventually underwent a further change. On continued passage the growth rate declined to a low level, and the cultures eventually became static for 2–3 weeks, though without showing a cytopathic effect. At this stage local foci of piled up cells appeared, consisting of highly basophilic "blastoid" round cells. At their first appearance the latter seemed to be "nested" within the cytoplasm of an attached cell, for they had a clear halo around them, which could be enhanced by the use of phase-contrast illumination (Fig. 4a). This did not seem to be the result of phagocytosis, because debris or degenerating cells were not found in this situation; moreover, many of the rounded cells could be seen to be in mitosis, while still within the cytoplasm of the reticular cells (Fig. 4b). At an intermediate stage of "transformation", practically every reticular cell could be seen to have one or more round cells within it. The question remains open as to whether the round cells actually arise within the reticular cells, or enter the latter's cytoplasm to complete their development.

This process of "blastoid transformation" continued for 4–5 weeks, when the cultures from one patient (Table 1, No. 5) had become entirely composed of free floating cells (blastoid

cells) (Fig. 5) with large variations in cell size and number of nuclei; at the same time the growth rate increased approximately eight-fold, considerably exceeding that of the parent culture. Transformation occurred on two different occasions with separate samples from this same patient, each at about the same time in the history of the resultant cell line. On the first occasion transformation occurred after lymph node fragments had remained for 1 month on the grid, followed by nine transfers as monolayer cultures. The second time the fragments were on the grid for 4 months, followed by six monolayer passages. A single stock of reticular cells from one of these cell lines before transformation was kept stored at -78°C ; on four later occasions, when cells from this stock were thawed out and subsequently propagated, complete transformation into blastoid cells took place. Cells from a second patient (2) also gave rise to blastoid cells of the type described, but in this case they could only be propagated by using a feeder layer, and thus resembled those of Pontén⁹. At the time of writing, attached cell lines from three more patients (6, 7 and 8) are beginning to undergo similar changes.

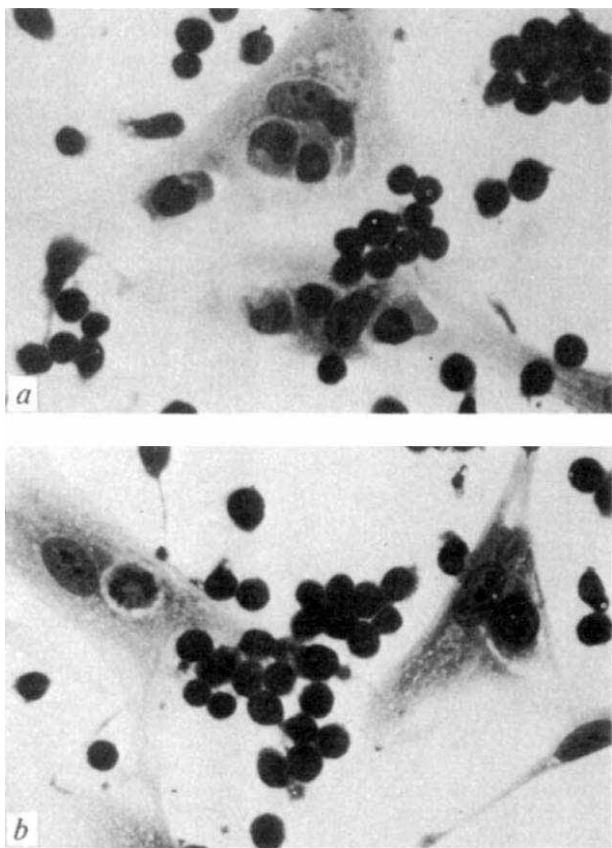


Fig. 4 a, "Blastoid transformation" intermediate stage ($\times 384$). Note cells nested within the cytoplasm of settled cells; multinucleate giant cell. b, Further advanced "blastoid transformation" ($\times 384$). Note group of cells in mitosis.

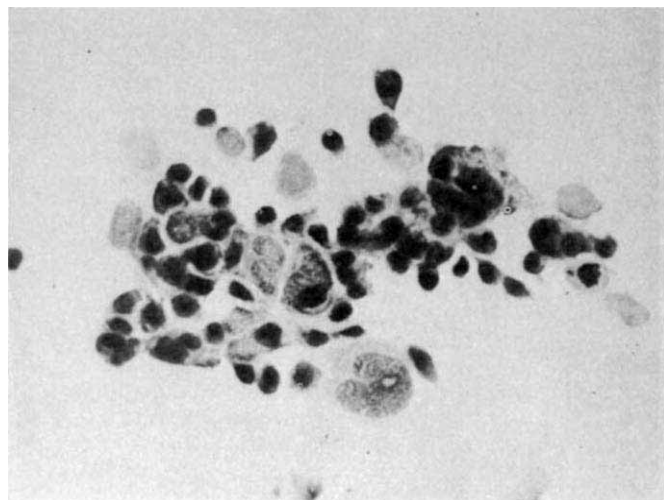


Fig. 5 Cells from suspended culture of transformed cells. Note large variations in cell size and nuclei ($\times 375$).

At least three types of blastoid cells could be distinguished (Fig. 5): (a) small, highly basophilic cells with a large round nucleus; (b) medium sized, less basophilic, sometimes vacuolated cells, with a large nucleus containing multiple irregularly shaped nucleoli; (c) multinucleated giant cells with many nucleoli. The floating cells generally also had forty-six chromosomes although two out of forty-one cells from the floating cell line derived from patient 5 also lacked the Y chromosome; a notable feature of these cells was the elevated frequency of enhanced secondary constrictions of chromosomes 1, 9 and 16.

The blastoid cells differed strikingly in ultrastructure from the attached cells. Their nuclei showed more condensed heterochromatin and larger numbers of perichromatin granules, and throughout their cytoplasm mitochondria with a dense matrix and conspicuous "mitochondrial ribosomes" could be seen. No boundary was apparent between ectoplasm and endoplasm, and the microfilaments, so conspicuous in the reticular cell lines, were barely visible. Moreover, the endoplasmic reticulum was poorly developed, and its cisternae contained no accumulated secretion product. Most ribosomes appeared as "free" monosomes, while in the reticular cells most of them were attached to membranes in typical "polysome" patterns. Clumps of blastoid cells showed many examples of close contact of their plasmalemma, giving the appearance of tight junctions. Thus while the reticular cells have some of the

ultrastructural features of histiocytes, the floating blastoid cells resemble blastoid elements of the haemopoietic system.

Release of Agent containing DNA

Labelling experiments were also performed with one of the suspended cell lines that arose following the "blastoid" transformation just described (patient 5). The technique was the same as that used with the settled cell lines. $5\text{-}^3\text{H}$ -uridine still became incorporated into material banding isopycnicly on sucrose at 1.20–1.22 (Fig. 6). But when ^3H -thymidine instead of $5\text{-}^3\text{H}$ -uridine was used, a strongly labelled DNA-containing peak was consistently found banding at the same position in the gradient (1.20–1.22; Fig. 7). At the outset, it was suspected that both the DNA and RNA-containing bands might be due to concurrent mycoplasma contamination, and bacteriological examination revealed that this culture line was indeed infected with mycoplasma. The following evidence, however, makes it unlikely that mycoplasma was responsible for this band.

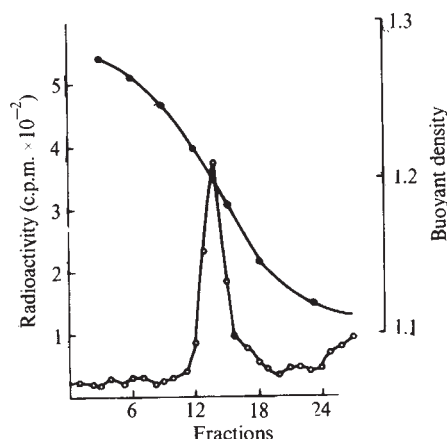


Fig. 6 Equilibrium density centrifugation in 20–58% w/w sucrose of pelleted ^3H -uridine-labelled material from supernatant fluid of "transformed" suspended cell culture SK-HNF-5b (16 h, 27,000 r.p.m., SW.27, 5°C). Symbols as in Fig. 2.

(a) Mycoplasma, isolated from the culture line itself, grown up in broth and concentrated by centrifugation, banded on sucrose at an equivalent density of 1.24–1.26, many fractions further down the gradient than the labelled band mentioned above. (b) Cultures of FL amnion cells and WI-38 fibroblasts were deliberately infected with a high level of this same mycoplasma, and then labelled with ^3H -uridine in the manner described. The tissue culture fluids were then processed in the same way as before, and the concentrates banded isopycnicly on sucrose. No labelled peak was seen. (c) The mycoplasma-contaminated "blastoid" cell line was treated by Dr J. Fogh until there was no longer any evidence of mycoplasma. ^3H -thymidine labelling was undertaken, this time in another laboratory known to be free from mycoplasma, and the fluids examined. A ^3H -labelled peak at 1.20–1.22 was again present, of greater size than before. (d) Electron microscope examination of thin sections of the pelleted supernatant revealed the presence of double-enveloped nucleocapsids typical of the herpesvirus group. (e) There was also immunological evidence—to be discussed in the next section—of the presence of herpesvirus antigens, both in the cells and in the material from the sucrose band.

Serology of DNA containing Agent

Serological studies of the SK-HNF-5 cell line are being conducted in collaboration with Miss Gayla Geering and Drs Germaine Trempe, T. Takahashi and Lloyd Old. As expected from the morphological evidence of the presence of an asso-

ciated herpesvirus, herpesvirus gs antigen has been demonstrated in the pelleted virus. Immunodiffusion and immunofluorescence studies have failed to show EB virus-related antigen in the SK-HNF-5 cell line; however, a new antigen has been found in the cell line by immunodiffusion, and precipitating antibody to this antigen is found in the sera of a high proportion of normal human subjects.

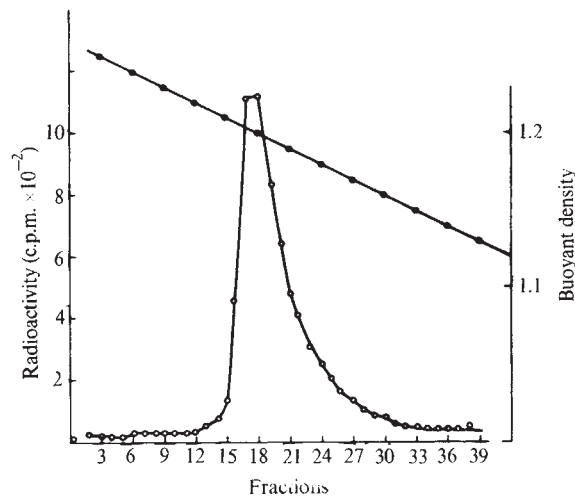


Fig. 7 Equilibrium density centrifugation in 20–58% w/w sucrose of pelleted ^3H -thymidine-labelled material from supernatant fluid of "transformed" suspended cell culture SK-HNF-5b (16 h, 27,000 r.p.m., SW.27 rotor, 5°C). Symbols as in Fig. 2.

Our findings suggest that the process of "blastoid transformation" in this case may have been accompanied by the emergence of an agent of the herpes group. Further experiments are in progress to discover whether a similar phenomenon occurred during the other instances of transformation reported.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Eclipsing Binary Model of Cygnus XR-1

AGRAWAL *et al.*¹ present an interesting observation of the variable X-ray source Cygnus XR-1 at energies above 20 keV. This communication points out that their observations may be consistently interpreted in terms of the eclipsing binary model for the source that was originally outlined in an article by myself² (article 1).

Agrawal *et al.* observed the source in nineteen consecutive periods of approximately 5 min from 0002 to 0315 UT, on April 6, 1971. The mean count rate in the 22.5–99 keV energy range for periods 1 to 12 was 260 counts per min (although non-random variations in the count rate appear to be present) and there was “no significant variation” in this source count rate for the following four periods (13 to 16). The three succeeding periods, however (17 to 19), show “considerably lower values” of source count rate; the authors infer from these results the fact that the source “decreased from the mean value corresponding to (periods) 1–12 by a factor of ~ 2 . The observations (between periods 16 and 17) are consecutive in time and therefore the fall in intensity has taken place within a few minutes”.

According to the eclipsing binary model outlined in article 1, Cygnus XR-1 is composed of a source of small angular extent (either a dense star or a small, optically thin region associated with it) having a spectrum similar to that of a black body at $kT=12.5$ keV and a non-X-radiating companion which eclipses it at intervals of 2.9850 d. The system is surrounded by an X-radiating plasma the photon flux of which between 1 and 100 keV can be approximated by a power law spectrum the exponent of which is -1.7 . Using an epoch of (primary) minimum derived in article 1 of JD 2 439 382.71 [which is uncertain to something like 1 h], a minimum would thus be predicted to occur at JD 2 441 047.88. Because the eclipse minimum was shown in article 1 to last at least 0.66 day but less than 0.80 day, this would predict an occultation of the black body source sometime between 2330 and 0115 UT on April 5–6, 1971. If we accept the discrepancy of 1 h as being caused by the uncertainty in the epoch of primary minimum, it is possible to interpret the observations of Agrawal *et al.* as being caused by the immersion of the black body source behind its non X-radiating companion. To support this interpretation we note first that the variation occurs in the right direction, that is, a decrease in flux before the time of minimum, second that the flux decrease in the 22–99 keV range is of the amount ($\sim 1/2$ the maximum flux observed from Cygnus XR-1) ascribed to the black body source in the eclipsing model, that is, it is the amount which, added to the state of minimum flux defined in article 1, would produce the state of maximum flux. Third, no effect was seen over the

same time period in the energy region 99–154 keV by Agrawal *et al.*¹. Using the data in their Table 1, the 19 observations had a χ^2 of 19.5; 39.2% of all random distributions are more random than this for 18 degrees of freedom. Thus, the observation in this energy region is consistent with that expected from the random arrival of photons from a non-varying source, which in the eclipsing binary model would be identified with the X-radiating plasma surrounding the system. The black body source intensity above 100 keV, of course, is negligible with $kT=12.5$ keV. Fourth, the total immersion of the black body takes place over a short time period, that is, over a period of minutes. This is the expected time of occultation of an object of a few kilometres diameter at a distance of ~ 1 kpc in an orbit of period 3 days about a solar size object. If the flux difference between the states of maximum and minimum flux is taken to be $\sim 1.5 \times 10^{-8}$ ergs cm^{-2} s, then the distance of the 1.5×10^8 K black body producing this flux is $0.9 R$ kpc, where R is the radius of the black body in kilometres. The irregularity in the flux immediately before immersion is perhaps related to the line of sight passing through a region near a distorted stellar atmosphere the boundary of which is not sharp in the sense of the solar limb.

Since the publication of article 1, three other observations of Cyg XR-1 have been made in the region above 20 keV. Weber and Reinert (private communication) observed the source on JD 2 440 352.87 and obtained a spectrum consistent with a state of minimum flux. The nearest minimum predicted in article 1 was at JD 2 440 352.83, consistent with the observation. Haymes and Harnden³ observed the source between JD 2 440 377.78 and 378.07; their resultant, high quality spectrum is nearly coincident with their previous measurement of the object in the state of maximum flux. The nearest minimum predicted in article 1 occurs at JD 2 440 376.71, 1.22 days before, and so is consistent with the observed state. Agrawal *et al.*⁴ also observed Cyg XR-1 between JD 2 440 328.36 and 328.47; the spectrum they derived would be classified as “uncertain” (with regard to the level of flux) according to the criteria outlined in article 1. Because the nearest minimum is predicted to occur at JD 2 440 328.96, this observation must have occurred at a time of maximum intensity.

Observation of Cyg XR-1 from the satellite Explorer 42 (ref. 5) in the 2.4 to 6.9 keV region indicated that approximately 25% of the flux exhibits short time variability. Although no eclipse phenomena seem to be present in this energy region (see article 1), this observation clearly indicates the multiple source nature of the object. The observation of the variable source Cen X-3 from the same satellite⁶ seems to indicate the same type of pulsation observed from Cyg XR-1 in this energy range. Since Cen X-3 is best fitted by a black body spectrum of 3.3×10^7 K, it is tempting to interpret the smooth variation in flux level in Fig. 1 of ref. 6 as the immersion of an eclipsing binary system. The lower temperature of the black body and the less intense continuous radiation relative to it make the variability more evident for this source in the satellite's lower energy range of observation.

The eclipsing binary model for the variability of Cyg XR-1 as presented in article 1 thus still seems to be consistent with all observations of the object so far.

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Optical Identification of Cygnus X-1

THE star HD 226868 is coincident, within a 3" error, with a faint and possibly variable radio source which itself is coincident, though with a large uncertainty, with the Cygnus X-1 pulsating X-ray star¹.

Table 1 Data on HD 226868 (= BD 34° 3815)

$V = 8.89$; $(B - V) = +0.85$; $(U - B) = -0.24$ (Hiltner ³)	
$S_p = \text{BOIb}$ (Morgan, Code and Whitford ² ; RGO)	
$V_r(*) = -12 \text{ km s}^{-1}$ (Seyfert and Popper ⁸)	
$V_r(K) = -24 \text{ km s}^{-1}$	
$= -13 \pm 7 \text{ km s}^{-1}$ (RGO)	
Equivalent width of K line	
$= 0.3 \text{ \AA}$ (Seyfert and Popper ⁸)	
$= 0.6 \pm 0.1 \text{ \AA}$ (RGO)	
Central depth of diffuse interstellar line (4430 \AA)	
$= 9 \pm 2\%$ (RGO)	
$Q = -0.89 \text{ mags}$	
$E_{(B-V)} = 1.07 \text{ mags}$	
$m_0 - M = 11.5 \text{ mags}$	
$d = 2.0 \pm 0.5 \text{ kpc}$	

Table 1 lists information about HD 226868 obtained from the literature and from spectrograms taken by observers at the Royal Greenwich Observatory (RGO) with the image tube and photographic spectrographs attached to the 98 inch telescope; the measurements were made between July 26 and August 4, 1971. The RGO spectra were at dispersions of 60, 70 and 180 \AA mm⁻¹ over the spectral range 3600 to 5200 \AA. The star appears to be a normal BOIb supergiant as classified by Morgan *et al.*² (The visibility of the high Balmer lines in HD 226868 is slightly less than the visibility of those in a comparison star HD 192422, BO.5Ib, which tends to decrease its luminosity towards II.)

The allocation of the spectral type BOIb is supported by the photoelectric colours of Hiltner³ which give

$$Q = (U - B) - 0.72(B - V) - 0.05(B - V)E_{B-V} = -0.89$$

agreeing precisely with the standard value⁴.

The spectroscopic distance modulus of HD 226868, calculated using $M_v = -5.8$ (ref. 5) and the value, $R = 3.0$, for the

ratio of total to selective absorption in this region⁶, is 11.5 mags, corresponding to a distance of 2.0 kpc. The chief uncertainty in the distance modulus arises from cosmic dispersion in the absolute magnitude of the spectral type, and corresponds to an error in the distance of ± 0.5 kpc. The intensities of the interstellar K-line and 4430 \AA diffuse band are consistent with the amount of reddening and the distance to the star. The kinematic distances of the star and K-line absorption are liable to large uncertainty because the star is near the null points of the galactic velocity field, but they are also consistent with the spectroscopic distance modulus.

The distance of 2.0 kpc which we ascribe to the star is, apparently, inconsistent with the upper limit of 1.0 kpc placed on the distance of Cygnus X-1 by Gursky *et al.*⁷ from the low energy turn-off in its X-ray spectrum due to photoelectric absorption in the interstellar medium. But because of the tentative identification of the supergiant with Cyg X-1 and the uncertainties expressed by Gursky *et al.* about the accuracy of their method, we feel it premature to attempt to save the phenomenon. If the double coincidence of position between the Cyg X-1 source, the radio star and the supergiant is too strong to be by chance, the X-ray source may be a companion to the supergiant, rather than identical to it. If the X-ray source has an optical absolute magnitude of +4, like the Crab Nebula pulsar, its optical luminosity is 10⁻⁴ times that of the supergiant. A distance of 2.0 kpc would imply that the observed power output in the X-ray region (0.5 to 100 keV) would be more than 10³⁷ erg s⁻¹ and comparable with the Crab Nebula.

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Possible Black Hole in Beta Lyrae

β LYRAE is one of the most controversial and puzzling of binary systems¹. The central issue concerns the absolute masses of the two components. The one-spectrum radial-velocity curve does not reveal what those masses are, but any pair of masses satisfying its mass function make the system remarkable. Table 1 gives pairs of masses which satisfy the observed 8.5 $M_\odot$ mass function². Here $q = M_{\text{BRT}}/M_{\text{FNT}}$, and the entries in the table are the minimum masses allowable—that is, the orbital inclination angle is assumed to be 90°. Early investigators, faced with the absence of the secondary component in the spectrograms at all phases, assumed that the components of the system obey a mass-luminosity law and favoured a mass ratio considerably larger than unity. This, however, results in very large masses, especially for the B8 primary. Three arguments have since been put forward which make the choice $q > 1$ dubious. First, Sahade³ has called attention to the behaviour of the unusual emission features present in the spectrograms, noting that, should they be asso-

ciated with the secondary star, a value for the mass ratio considerably less than unity results. Second, a similar small value comes from a distance determination by Abt⁴, which was based on β Lyrae's co-motion with few stars. As these were found to be ordinary main sequence objects, the absolute magnitude of β Lyrae was determined to be $M_v = -3.4$, which agrees with the spectroscopic luminosity class. So modest a luminosity can hardly be associated with such a massive primary component as indicated for $q > 1$. Finally, Woolf⁵ has used Abt's distance in combination with an assumed brightness temperature to find the absolute area of the primary star and thus its absolute "radius". Further, assuming that β Lyrae A fills its Roche lobe, this "radius" is compared to the observed value of $a_1 \sin i$ to obtain the mass ratio of the system, again giving $q < 1$.

Table 1 Minimum Absolute Masses which Satisfy the Observed Mass Function

$M_{\text{BRT}}/M_{\text{FNT}}$	M_{FNT} (solar masses)	$M_{\text{BRT(BB)}}$ (solar masses)
0	8.5	0
1/4	13.3	3.3
1/2	19.2	9.6
1/1	34.0	34.0
2/1	76.5	153.0

It is difficult to accept such a small mass ratio for the system, however, because the secondary must then be extraordinarily underluminous for its mass. Nevertheless, I shall show that this is a necessary consequence of the present observational evidence.

Although spectroscopy does not reveal the mass ratio of β Lyrae's components, the photometry of the system provides vital information through the photometric effect of tidal and rotational distortion. The aim of this article is to use this effect to determine the mass ratio by the method of fitting theoretical light curves to the observations throughout the phase interval between eclipses. The principle is straightforward, yet the method has not had widespread application. One reason is that the mass ratio is only one of several parameters which influence the out-of-eclipse variation and thus it is usually weakly determined. Furthermore, it has been difficult until recently to carry out calculations on the light changes of stars which display such severe photometric distortion as β Lyrae. The calculations presented here are adapted from a program for computing monochromatic, complete light curves of close binaries in which all the distortion and mutual irradiation effects are considered. The computer program and its application are presented in detail elsewhere⁶.

I shall show that β Lyrae is a most interesting exception in which the photometric technique can be applied to the determination of the mass ratio. Two factors are responsible for this. First, the primary (more luminous) component is in contact with its Roche lobe, a fact made evident by the pattern of gas-streaming in the system, pointed out by Struve¹ and also by Sahade³. Thus its relative size is uniquely determined by any assumed value of q . Second, this component is the only one which contributes significantly to the observed light variation between eclipses. The secondary may thus be neglected in the theoretical calculations, reducing considerably the number of free parameters, and elevating the mass ratio to the role of the dominant parameter. Because the neglect of the faint component is important to the application of the method, its justification will be considered in some detail, using information from the well known spectroscopic data and the recently published light curve of Larsson-Leander⁷, which resulted from the 1959 IAU International Campaign on β Lyrae.

The principal feature of the spectroscopy of this system is

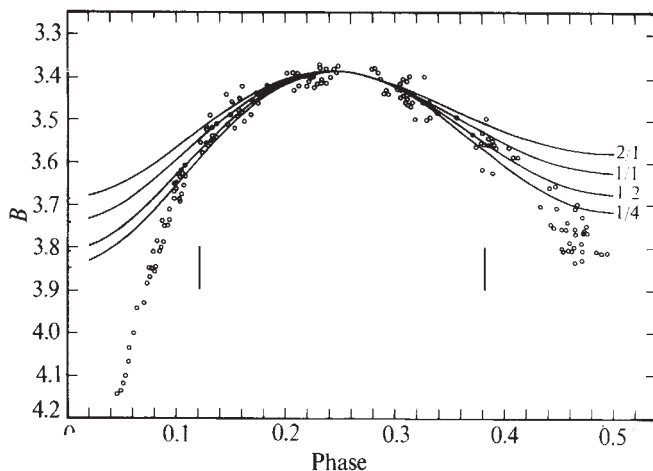


Fig. 1 The B light curve of Larsson-Leander with the theoretical curves as solid lines, given for labelled values of the mass ratio $M_{\text{BRT(BB)}}/M_{\text{FNT(INV)}}$. Other required parameters for the calculation are given in the text. The primary eclipse egress is shown and observations in the range of phases from 0.5 to 0.89 have been reflected about phase 0.5.

the total absence of an ordinary absorption spectrum attributable to a secondary component, even at mid-primary eclipse. At this phase, the light level drops to 48% of its value at the quarter phases. As the absence of one member of a binary from spectrograms is commonly taken to indicate that a factor of at least six separates the luminosity of the stars, it may be concluded that the secondary is responsible for no more than 8% of the light of the system. (The $B-V$ colour curve is quite flat so that this statement is true for either yellow or blue light.) Now consider the light curve shown in Fig. 1. It is noteworthy that the phase of external tangency, marking the end of eclipse, is visible clearly at a phase of 0.11, from which it can be seen that the amplitude of the eclipse effects reaches 0.20 magnitudes. A secondary with 8%, or less, of the system's light cannot be responsible for such changes. In fact, careful consideration shows that what the observations do reveal, with considerable fidelity, is the tidal and rotational distortion of the primary component alone. This can be seen by correcting the observations for the presence of a supposed 8% secondary, allowing, for purposes which will become evident, that it is somewhere in the distortion range between a perfectly spherical and a fully distorted or contact star. Treating the spherical case first, it is merely necessary to subtract 8% light from the observations at all phases. This results, on a magnitude scale, in a slight increase in the amplitude of the out-of-eclipse light variation amounting to 0.015 magnitudes. As the secondary is allowed to be more distorted, it is found that the corrected data approach more closely the original observations.

The theoretical calculations thus give the light variation of one contact component only, as a function of phase. The computations depend on just four parameters, in addition to the mass ratio. As the mass ratio sets the size of the contact primary in this case, the inclination angle is the only other geometrical parameter required and it has been assumed to be 90° . The coefficient of limb darkening was taken as 0.60, somewhat larger than theory predicts. The gravity-darkening law was that for an atmosphere in radiative equilibrium (von Zeipel's law). An effective temperature of 11,000 K was assumed, but the calculations are not sensitive to this parameter. The choice of 90° inclination, radiative equilibrium, and a coefficient of limb darkening larger than expected, as well as the neglect of a possible light contribution by the secondary, all insure the greatest light variation possible for any given mass ratio. This means that an upper limit for the mass ratio $M_{\text{BRT}}/M_{\text{FNT}}$ is found here. The results of the

theoretical calculations for selected values of the mass ratio are shown as solid curves in Fig. 1. It is evident that the primary has the minor share of the system's mass, and that very likely $q \approx 1/2$ is a realistic upper limit. If the secondary happens to be spherical, and contributes the maximum amount, 8%, to the light of the system, then it is conceivable that the mass ratio is a factor of two smaller. A significantly smaller mass ratio would also be appropriate if the primary could somehow have a convective atmosphere. It is also conceivable that the system's inclination angle differs so significantly from 90° that further reduction would be needed. Thus it is seen that the primary must not have more than $\sim 35\%$ of the mass of the system.

For $q = 1/2$, the absolute masses resulting for the primary and secondary are approximately $10 M_\odot$ and $20 M_\odot$ respectively. The mass of the primary is in good agreement with that determined by Abt from the slightly evolved position of β Lyrae A on the colour-luminosity diagram, but perhaps the evolutionary state of the star is also a function of its duplicity and the star may not be in equilibrium. It is now virtually certain that the secondary must be a most remarkable object, at least four magnitudes underluminous for its mass. Such a large value for its mass excludes the possibility of its being a white dwarf or neutron star, but it may be a black hole, or perhaps some previously unknown stellar state.

The photometric data furnish no information that the secondary is a collapsed object. In fact, there is clear indication of a geometrical eclipse in which about $1/3$ of the primary is hidden, so the eclipsing body must be substantial in size, yet it must be within its Roche critical surface, if the primary is at its own lobe. This is because the principal eclipse is over at a phase of 0.11, whereas the eclipse of two contact components would continue until a phase of 0.16 regardless of the mass ratio⁸. It is quite likely that the agent responsible for the primary eclipse is a disk, the existence of which is given strong credibility by the polarimetric work of Rucinski⁹. There also certainly appears to be a light loss due to eclipse at the secondary minimum. This loss does not seem to be due to the eclipse of the light of the primary reflected from the disk because then the reflexion effect of the system would have to be quite pronounced, which is not at all the case, so it is conceivable that the secondary itself is actually eclipsed. It would probably be meaningless to use the eclipse light loss at the two minima to infer the surface brightness ratio of the two components, because there exists the likely contribution of the disk to the principal minimum. Despite these difficulties in interpretation of the complete light curve of this star, the results for the mass ratio obtained here cannot be vitiated unless one is prepared to believe that the light variation between eclipses has some unknown and mysterious source. In view of the strong evidence for the primary being at its Roche lobe and also dominating the light of the system, the basis for such an idea is totally lacking.

On the contrary, the evidence is conclusive for the presence of a massive, underluminous object in the system of β Lyrae and we should probably look to it for an explanation of the remaining problems of the light curve.

An important item of evidence furnished by the identification of a black hole in a close binary system is that the collapse must have taken place without much mass loss because the system was not disrupted; $20 M_\odot$ must be approximately the mass of the pre-collapse object. It can also be suggested that the disk about the secondary was created by a mass-shedding process during collapse. As the secondary is invisible because of its small size, there is no reason to consider that the disk is an opaque, star-hiding body¹⁰, having a considerable fraction of the mass of the system⁵. The stability of such a ring would be questionable, so it is probable that only a small fraction of the mass of the collapsing object was lost to the disk.

The importance of this system for understanding the nature of massive, underluminous stars is great, and it is necessary to encourage all pertinent observations. Finally, it is ironic to

note that β Lyrae, the prototype of all β Lyrae systems, is not a β Lyrae system.

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Compact Radio Sources in the Galactic Nucleus

SMALL-SCALE radio structure within Sgr A, the source identified with the galactic centre, is of great interest because of the recent discovery of similar structure in the infrared¹ and because of current speculation on black holes in the galactic nucleus². In this communication we describe new observations

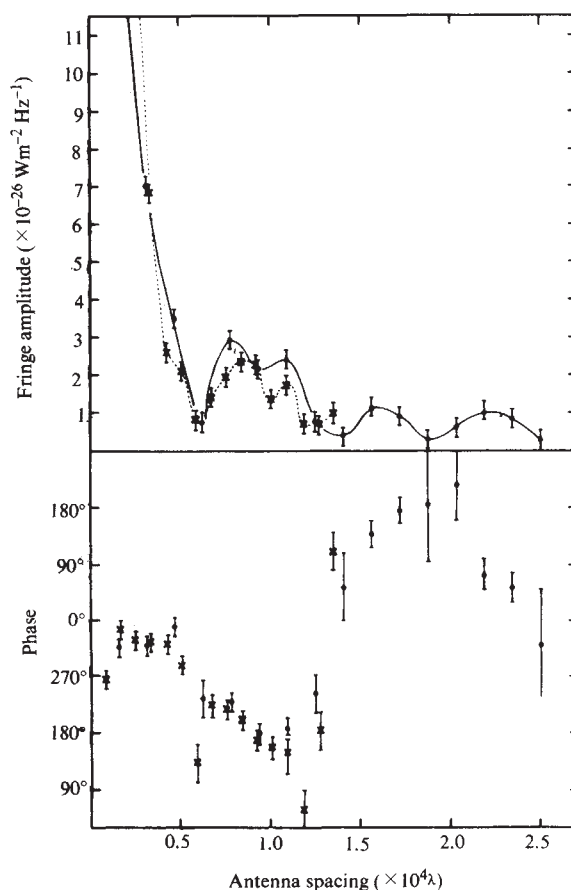
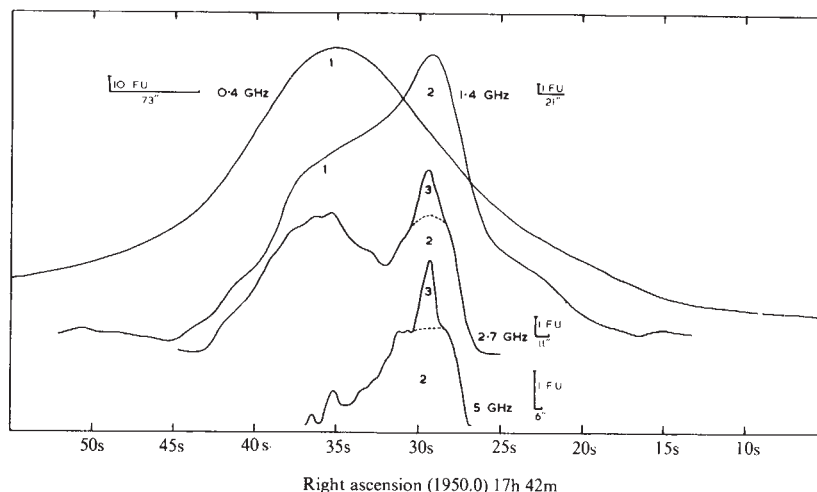


Fig. 1 Fringe amplitude and phase for Sgr A at transit at (x) 2.7 and (●) 5 GHz.

Fig. 2 Strip brightness distributions in right ascension for Sgr A at transit at 0.4, 1.4, 2.7 and 5 GHz. Horizontal bar indicates beamwidth, vertical bar indicates intensity of a point source. The components are numbered as in Table 1.



of Sgr A with the Cambridge 1 mile telescope at frequencies of 2.7 and 5 GHz. The beamwidths in right ascension were respectively 11 and 6 arcsec, the best of these being the highest resolution yet applied to the galactic centre. The observations suggest a three component model for Sgr A, and confirm the existence of radio structure on a scale of 10 arcsec. The relation of these compact radio sources to the infrared emission is also examined.

The observations were made between January and March 1971 at centre frequencies of 2.6970 and 4.9938 GHz, with sixteen antenna spacings along an east-west baseline. At each frequency the system temperature was about 1,000 K and the infrared bandwidth was 4 MHz. All observations were made

interferometric observations of the source. At spacings $>4000 \lambda$, the new visibility function shows the presence of fine structure in the source at even the longest spacing. The existence of fine structure was originally recognized by Ekers and Lynden-Bell (private communication) who used the interferometer at the Owens Valley Radio Observatory.

Before Fourier inversion of the observations, the fringe amplitudes were multiplied by a Gaussian weighting function which fell to a value of 30% at the largest spacing; this weighting function reduced sidelobes to 5% or less. The synthesized fan beam was centred on declination $-28^\circ 59' 30''$, and the beamwidth in declination was 16 and 9 arcmin at 2.7 and 5 GHz respectively.

Table 1 Model Proposed for Sgr A

Component name	r.a. 1950	dec. 1950	Halfwidth in r.a.	Flux density ($\times 10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$)			
				0.4	1.4	2.7	5.0 GHz
1 Sgr A East	17 h 42 m 36 s ± 1 s	$-28^\circ 59' 00'' \pm 60''$	$150'' \pm 30''$	250 ± 50	160 ± 30	> 80	—
2 Sgr A West	17 h 42 m 29.3 s ± 0.3 s	$-28^\circ 59' 15'' \pm 50''$	$45'' \pm 5''$	—	30 ± 5	25 ± 5	25 ± 5
3 Compact structure*	17 h 42 m 29.3 s ± 0.1 s	$-28^\circ 59' 20'' \pm 30''$	$10'' \pm 2''$	—	—	3 ± 1	3 ± 1

* Only the strongest feature has been tabulated.

during ± 1 h of meridian transit, with the antennas pointing 8 arcmin north of the true declination of the source to allow for atmospheric refraction. The primary source for calibration of phase and intensity was 3C 147, which was assumed to have flux densities of 13.0 and 8.2 flux units at 2.7 and 5 GHz respectively. The data were averaged for intervals of 15 min, for an r.m.s. sensitivity of 0.3 flux units in the fringe amplitude. In our experiment, the telescope produced a principal response whose half-width in right ascension was 11 and 6 arcsec at 2.7 and 5 GHz, with first grating lobes separated from the main beam by 4.1 and 2.2 arcmin in right ascension respectively.

Previous observations of this type³⁻⁵ have provided curves of fringe visibility and phase for Sgr A up to baselines of 2000λ at frequencies ~ 1 GHz. The maximum baseline of the present observations is $25,000 \lambda$ at 5 GHz. The fringe visibility and phase from the new observations are shown in Fig. 1 for the galactic centre at transit. At spacings $<4000 \lambda$ the curves show the tail of the response to the main part of Sgr A, which has an angular diameter ~ 3 arcmin. This is the part of the visibility function that has appeared in previous

The resulting brightness distributions are shown in Fig. 2. The new data at 2.7 and 5 GHz are shown together with earlier scans obtained at 0.408 and 1.407 GHz with sixteen spacings of the same telescope⁶. The profiles clearly show the shift in position of the peak of the source as noted by other observers⁶⁻⁹ and also show the existence of a prominent small diameter source at higher frequencies. The profile at 5 GHz, however, does not show structure of low spatial frequency (that is, of angular size >2 arcmin) that may be present, because of the limited number of antenna spacings used.

Fourier transforms were also made of the data taken at local hour angles ± 00 h 45 m, which correspond to position angles $\pm 6^\circ$ across the source. A comparison of the three scans gives information on the declination of the various components and suggests a three component model of Sgr A which is summarized in Table 1. We emphasize, however, the uncertainties in this model which are a consequence of our limited coverage in the Fourier transform plane. In particular, there seem to be additional components of size <10 arcsec and intensity <1 flux unit, which we have not tabulated. Among these are the peaks at r.a. 17 h 42 m 35 s,

and other peaks or ridges to the east and west of the 3 flux unit source at 17 h 42 m 29.3 s.

We interpret the radio spectra of the components in Table 1 as follows. The 2.5 arcmin source at r.a. 17 h 42 m 36 s (Sgr A East) is probably non-thermal because: (i) it emits most of the flux, and the spectral index $\alpha < -0.25$ ($S \propto \nu^\alpha$) derived from lunar occultations¹⁰ refers chiefly to this component. The predominance of this source at 0.2 to 0.4 GHz explains the increase in diameter of Sgr A with decreasing frequency and also its position shift to the east; (ii) H109 α recombination line emission is at least an order of magnitude less than one would expect from a thermal source of this flux density¹¹; (iii) for a thermal source of this size and intensity to be optically thin at 200 MHz (ref. 10), the electron temperature would have to be $> 10^5$ K.

Both sources in the complex at 17 h 42 m 29.3 s, consisting of Sgr A West and the compact structure with diameter ~ 10 arcsec, appear to have flat spectra. At present we cannot distinguish between a thermal and non-thermal origin for the sources in this region. If they are thermal, then their linear sizes and flux densities are comparable with those of compact H II regions¹². The sources would become optically thick below 1 GHz and this would help to explain the position shift at low frequencies. If, on the other hand, these sources are non-thermal, they must simply have a flatter spectrum than Sgr A East. The same effects of diameter increase and position shift would then be observed in lunar occultations. On a synchrotron interpretation, the compact component would be similar to the bright radio knots seen in Cas A (ref. 13) and would have a minimum energy of 10^{46} erg.

On the basis of existing measurements, the group of infrared sources prominent at 5 to 20 μ m (ref. 1) is displaced by about 10 to 15 arcsec to the east of our component 3. None of the localized sources at these infrared wavelengths coincides with any of the radio components. This may be similar to the situation in some H II regions, for example, the Orion Nebula, where the IR nebula¹⁴ seen at 10 to 20 μ m does not coincide with the radio peak.

As the small components seen in Sgr A at both radio and infrared wavelengths have diameters of the order of a light year or less, it may be possible to detect intensity variations on a timescale of a few months. We also suggest that the source be monitored for interplanetary scintillation, which might reveal even smaller radio components in Sgr A.

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Reduction of Dinitrogen in Aqueous Solution

NITROGENASE, which contains iron, molybdenum and thiol groups, operates in an aqueous environment. Many attempts have been made to produce chemical models for nitrogenase based on the above knowledge and recently Schrauzer and co-workers have described dinitrogen-reducing systems of this type^{1,2} but they use high pressures of dinitrogen (135 atm) and obtain only 3 to 5 μ mol. of ammonia from mmol quantities of reagents² (see Table 1, example 1).

When the levels of reduction are very low and other nitrogen-containing ligands are present, it is essential to use ¹⁵N₂ to confirm the reduction of dinitrogen to ammonia. We have therefore tested with ¹⁵N₂ two of Schrauzer's systems and other systems which we have developed. Our quantitative

Table 1 Dinitrogen Reducing Systems

Reagents * † (mmol $\times 10^3$) and reaction times		Approximate yield of ¹⁵ NH ₃ ‡ (mmol $\times 10^3$)
(1)	1-Thioglycerol (2,500); Na ₂ MoO ₄ (5,000); FeSO ₄ (100); NaBH ₄ (6,600); 120 h, p ¹⁴ N ₂ = 135 atm	3 to 5§
(2)	Cysteine (20); Na ₂ MoO ₄ (40); FeSO ₄ (0.8); NaBH ₄ (290); 60 h	3
(3)	Cysteine (20); Na ₂ MoO ₄ (40); NaBH ₄ (290); 60 h	0
(4)	2-AET (60); Na ₂ MoO ₄ (60); FeSO ₄ (1); NaBH ₄ (390); 12 h	90
(5)	BSA (40,000)¶; Na ₂ MoO ₄ (20); FeSO ₄ (0.8); NaBH ₄ (290); 60 h	5
(6)	BSA (50,000)¶; FeSO ₄ (20); NaBH ₄ (290); 60 h	3
(7)	2-AET (20); Na ₂ MoO ₄ (40); FeSO ₄ (0.8); NaBH ₄ (290); 60 h, pCO = 0.5 atm	0
(8)	2-AET (20); Na ₂ MoO ₄ (20); FeSO ₄ (0.8); NaBH ₄ (290); H ₂ NC (CH ₂ OH ₃) (100); 60 h	0
(9)	V ²⁺ (100); Mg ²⁺ (5,000); KOH (56,000); 0.2 h; 70°	4**
(10)	Mo ³⁺ (40); Mg ²⁺ (180); Ti ³⁺ (300); KOH (12,800); 24 h	3 ‡ †

* p¹⁵N₂ ~ 1 atm; 30 ml. gas. † Final volume of aqueous solution ~ 1.5 ml. ‡ Mass spectral analysis of derived ¹⁵N₂. § Data from ref. 2, product is ¹⁴NH₃. || Compare ref. 2, where pCO of 0.1 atm does not inhibit. ¶ Milligram quantity $\times 10^3$. ** Reaction product is ¹⁵N₂H₄. ‡ † Solvent methanol/water (5 : 1).

2-AET = 2-Aminoethanethiol; BSA = bovine serum albumin.

results, some of which are negative, are given in Table 1, examples 2 to 10.

Schrauzer's system contains cysteine, iron, molybdenum and NaBH_4 (ref. 1) and undoubtedly gives traces of $^{15}\text{NH}_3$ from $^{15}\text{N}_2$ even at 1 atm (example 2), but not in the absence of iron (example 3). Working along parallel lines we have used 2-aminoethanethiol and sodium borohydride to develop a system which gives greatly increased yields of ammonia at pressures of 1 atm and reaction times of a few hours, that is, approaching the natural conditions (example 4).

High yields of ammonia are only obtained when iron is used together with molybdenum, but we have been able to replace the thiol by bovine serum albumin (a thiol-containing protein) and to obtain low yields of ammonia even when iron is the only added metal (compare examples 5 and 6); here molybdenum may be present at a trace level but added molybdenum alone is not effective. Low levels of dinitrogen reduction using iron complexes have been reported, but no experimental details have been given³.

High concentration of carbon monoxide inhibits our system (example 7) as does *tris*-hydroxymethylaminomethane—a buffer substance often used in the preparation of active extracts from the natural system (example 8)—presumably because it sequesters the iron from its active role.

We have also confirmed the results of Shilov and co-workers⁴ that $^{15}\text{N}_2$ is reduced to hydrazine- $^{15}\text{N}_2$ in strongly alkaline solution with molybdenum or vanadium as a catalyst (examples 9 and 10). There is thus no doubt that dinitrogen is reduced in these aqueous systems and further modification may yield useful systems for the catalytic activation of dinitrogen.

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Occurrence of Biogenic Siliceous Sediments in the Atlantic Ocean

ALTHOUGH the deposition of biogenic siliceous sediments is not widespread in the modern Atlantic and Caribbean, calcareous siliceous or siliceous sediments (including cherts) are recorded at various intervals in the Mesozoic¹⁻⁷ and Tertiary sequences^{1-5,7-10} of both areas. On the basis of the available data it appears that the deposition of these sediments was widespread in these areas during the Lower Tertiary and possibly the Mesozoic, and more restricted during the Upper Tertiary. In this article I describe and attempt to account for the distribution of these sediments.

There are unfortunately few data concerning either the stratigraphical or the geographical distribution of Mesozoic biogenic siliceous sediments. The discovery of radiolarian oozes and cherts at eleven localities investigated by the Deep Sea Drilling Project suggests that these sediments may have been deposited intermittently over a wide area during the Upper Jurassic^{1,5,6} and Upper Cretaceous (Cenomanian to Campanian)^{2-4,8}.

The distribution of biogenic siliceous sediments is better documented for the Lower Tertiary (see Fig. 1). Eocene

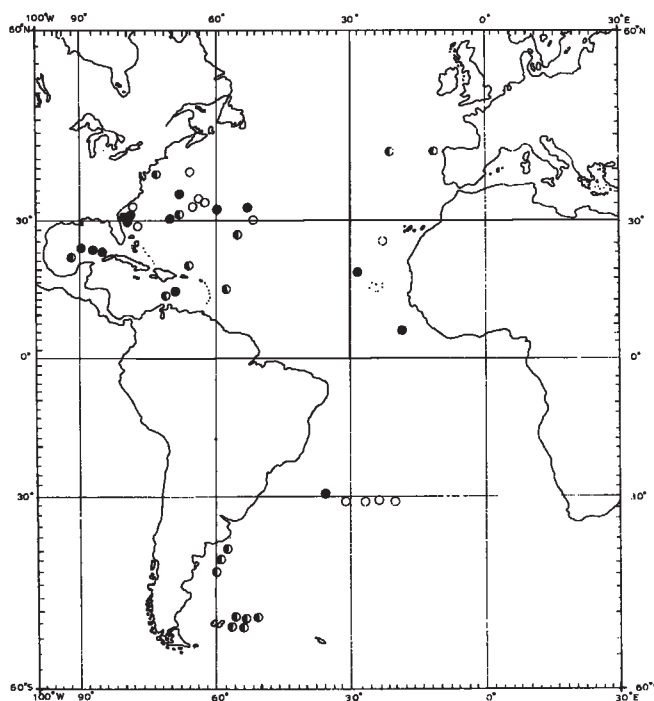


Fig. 1 The distribution of Eocene sediments in the Atlantic: filled circles represent chert, half-filled circles represent unconsolidated siliceous or calcareous siliceous ooze, and open circles represent calcareous ooze.

siliceous oozes and/or cherts are recorded at several widely separated localities, and in the North Atlantic the prominent and widespread reflector horizon A can at least in part be identified with Middle Eocene chert beds.

As a consequence of ocean floor spreading, the present location of the Palaeogene siliceous sediments does not conform with their position of deposition. Their distribution in the North Atlantic and Caribbean is evidently also unrelated to the present hydrography, biology and productivity of these areas. Fig. 2 shows the position of the Palaeogene localities plotted on Francheteau's¹¹ reconstruction of the Eocene Atlantic. On this chart two areas of biogenic siliceous sedimentation are defined: a southern zone situated along the western margin of the Argentine Basin, and a central equatorial zone. By analogy with the Recent oceans both areas were regions of upwelling characterized by a high production of plankton, and reflect the positions of silica belts established for the surface suspension during the Palaeogene. The southern area still lies within the major belt of silica accumulation which is associated with the Antarctic Convergence and West Wind Drift. Data presented by Saito and Funnell¹² show that this has been an area of opaline siliceous sedimentation since the Cretaceous. Although upwelling occurs at the equator in the Atlantic, the equatorial zone has no modern counterpart. In the Pacific Ocean, however, radiolarian ooze accumulates within a zone extending from 20° N to 20° S (ref. 13), which is associated with the equatorial current system. According to information published by Riedel¹⁴ and Riedel and Funnell¹⁵, this zone was already established during the Lower Tertiary.

The variation between the pelagic sediments associated with the Atlantic and Pacific equatorial current systems may be due to differences in their surface productivity which are caused by the higher concentration of phosphates, nitrates and silicates in the Pacific¹⁶. The low concentration of these nutrients in the equatorial Atlantic is directly related to the exchange of water between the North and South Atlantic, which results in their removal via the south flowing deep water of the North Atlantic¹⁶. On the basis of evidence obtained for Upper Cretaceous and Tertiary deep water circulation in the North Atlantic by Leg II of the Deep Sea Drilling

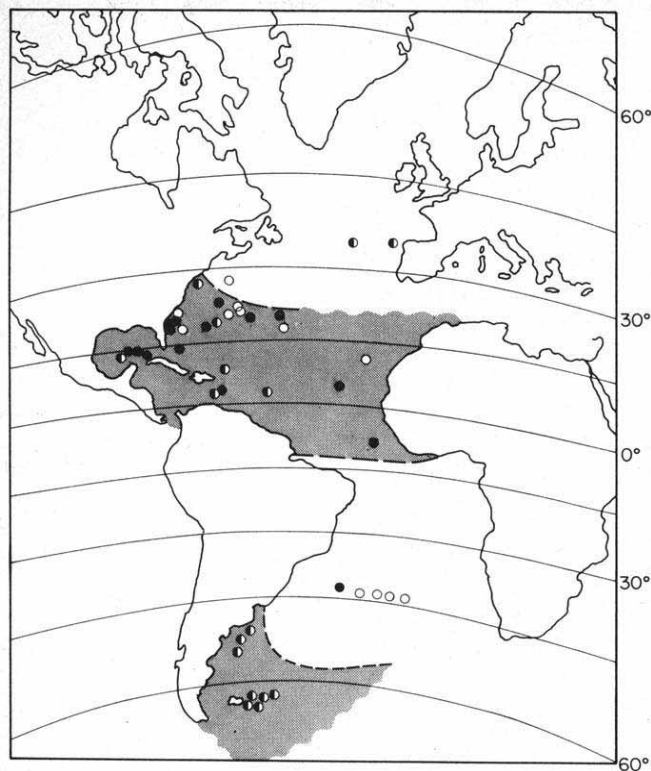


Fig. 2 The potential distribution of biogenic siliceous sediments in the Eocene Atlantic and Caribbean.

Project⁵, it appears that a similar process may have operated in the Lower Tertiary Atlantic. Consequently it is unlikely that an equatorial zone of biogenic siliceous sedimentation would have developed in the Palaeogene Atlantic unless there was an influx of water rich in nutrients into this area from an outside source. Such an influx could conceivably have been achieved by the flow of Pacific equatorial water across the Isthmus of Panama via the east flowing equatorial surface countercurrent and subsurface Cromwell current (see Fig. 3). As the continuity of the present countercurrents is apparently seasonal in either ocean¹⁷, the bulk of this flow was probably effected by the Cromwell current. At present this current is approximately 300 km wide and is symmetrical about the equator^{17,18}. It occupies depths between 50 and 200 m and has a total volume transport of about $40 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ (ref. 18). A depth greater than 200 m was therefore necessary to facilitate the passage of this current across the Isthmus of Panama during the Lower Tertiary.

The distribution of calcareous and siliceous or calcareous siliceous sediments on the Palaeogene ocean floor can be explained in terms of the circulation pattern proposed in Fig. 3. In this model the accumulation of calcareous ooze is confined essentially to areas of low productivity at the centre of the gyres. Siliceous or calcareous siliceous sedimentation occurred in regions of high productivity associated with areas of upwelling, or at the gyre margins. As in the modern oceans the accumulation of opaline silica in the bottom sediments can be attributed to the absence of a critical depth for its preservation, and to the resistance of radiolarians, silico-flagellates and the more robust species of diatoms to solution in the water column¹³. Also the increased production of carbon dioxide in sediments which underlie the fertile areas of oceans and the concomitant depression in the pH of the sea water^{19,20} result in a decrease in the solubility of opaline silica and enhance the preservation of siliceous skeletal material²¹ for its duration at the sediment-water interface. From the data presented in Figs. 2 and 3 it is apparent that the formation of the Eocene cherts in the North Atlantic is closely associated with the deposition of opaline silica in the

Palaeogene equatorial high productivity zone. In terms of the modern Atlantic the information contained in these charts suggests that the equatorial cherts should not be encountered south of 6° N and north of 36° N.

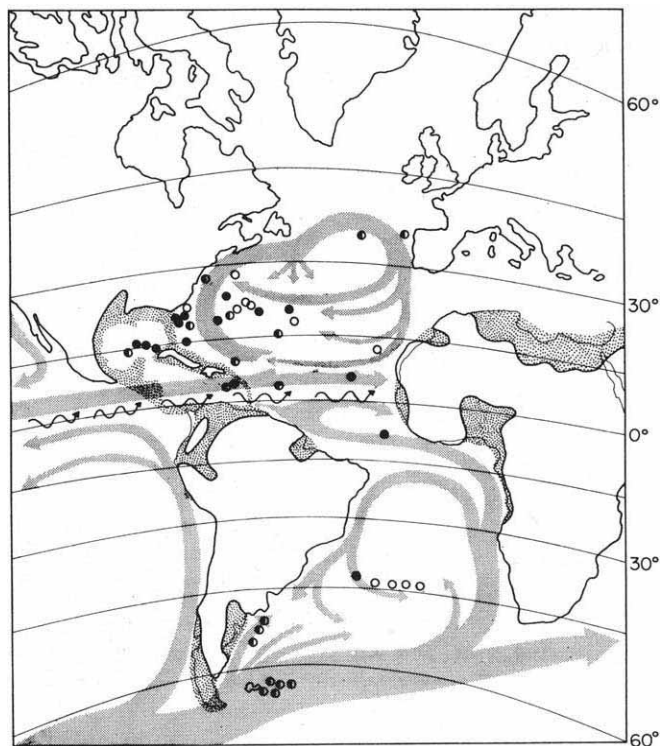


Fig. 3 Proposed circulation pattern for the Eocene Atlantic. The broad arrows represent surface currents, the sinuous arrow the Cromwell or equatorial undercurrent. The stippled regions on the continents represent shelf areas.

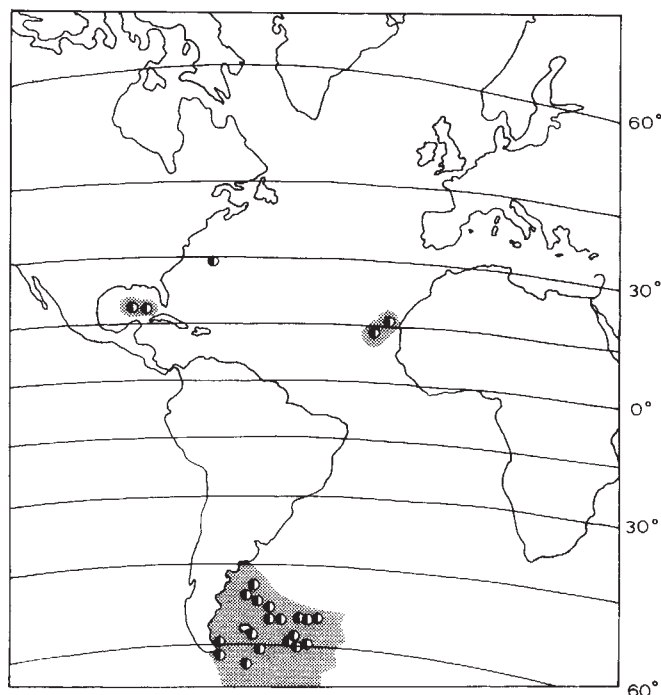


Fig. 4 The distribution of biogenic siliceous sediments in the Miocene Atlantic and Caribbean. The areas of primary production are stippled.

The equatorial zone of siliceous sedimentation had ceased to exist by the Miocene (see Fig. 4). The results of drilling and piston coring in the Atlantic and Caribbean^{4,5,7,12} show that during this epoch the deposition of opaline silica occurred primarily in areas of upwelling along the western margin of the Argentine Basin and along the west coast of Africa. In the Gulf of Mexico radiolaria which may have been derived from the Pacific flourished in what is still a region of high biological productivity²² and contributed to the formation of calcareous siliceous oozes. The distribution of biogenic siliceous sediments in the Miocene Atlantic suggests a pattern of surface and deep water circulation similar to the Recent.

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First Diagnostic Carboniferous Fossils from New Zealand

THE first New Zealand fossils indicative of Carboniferous age have been found in rocks from the South Canterbury region of the South Island. They are conodonts, accompanied by rare fish scales, and were obtained from the marble of the Kakahu inlier in a Tertiary area 10 miles south-west of Geraldine¹: fossil sample locality No. S102/607, grid ref. 627815 (latitude 44° 09' S, longitude 171° 04' 30" E).

The fossils were recovered from residues obtained by digestion of marble samples in acetic acid. The conodonts include some taxa which are known only from rocks of Pennsylvanian age, that is they are equivalent to Upper Carboniferous in North American terminology. The conodonts belong chiefly to the following genera, the time ranges of which as given by Hass² are appended: *Idiognathodus* Lower Pennsylvanian to Upper Pennsylvanian; *Cavusgnathus* Upper Mississippian to Lower Permian; *Gnathodus** Uppermost Devonian to Middle Permian; *Gondolella* Middle Pennsylvanian to Upper Triassic; *Hindeodella*? Lower Silurian to Upper Triassic; and *Ligonodina* Middle Ordovician to Middle Triassic.

The conodonts are opaque and whitish to dark grey in colour; many are distorted, no doubt as a result of the low grade regional metamorphism (chlorite 2 subzone) of the containing rocks. In spite of the distortion most specimens are generically determinable.

Specimens of *Idiognathodus* and *Cavusgnathus* are about equally common in the fauna as recovered. The remaining genera are represented by far fewer specimens; among these are specimens of *Gnathodus bassleri* (Harris and Hollingsworth) and *G. cf. roundyi* Gunnell. *Gondolella* is represented by a few incomplete specimens, mostly with unornamented platforms.

Ranges of represented genera indicate an upper Middle Pennsylvanian or lower Upper Pennsylvanian age for the Kakahu fauna. *Idiognathodus* seems to be absent from both the youngest and oldest Pennsylvanian faunas of North America; an abundance of *Idiognathodus*, as in the Kakahu fauna, is reported to be a characteristic of the Desmoinesian Stage (that is, upper Middle Pennsylvanian). *Gondolella* first appears in the Desmoinesian. On the other hand *Gnathodus bassleri* has been regarded by Lane⁵ as characteristic of the Lower Pennsylvanian (Morrowan), but in Nevada it is reported by Webster⁶ to range through the Derryan (that is, lower Middle Pennsylvanian). Further taxonomic work is necessary to resolve finally the question of the age of the Kakahu fauna within the Pennsylvanian.

Only indeterminate pelmatozoan remains and doubtful radiolaria had previously been recorded from the Kakahu rocks⁷; their age was therefore in doubt and suggested ages have included Lower Palaeozoic (Ordovician?)⁸, early Palaeozoic⁹ and late Permian, Lower Kazanian¹⁰. Discussing the upper Palaeozoic and lower Mesozoic rocks of South Canterbury and North Otago, Gair (in Waterhouse¹¹) suggested that the chlorite 2 subzone rocks of the Kakahu district could be "at least of Lower Permian or Carboniferous age". Gair subsequently included¹² the Kakahu Marble in the Haast Schist Group which he noted as grading into sediments of Permian and Triassic age belonging to the Torlesse Group. Wright mentioned¹³ having processed Kakahu limestone for conodonts in the course of his work on Ordovician conodonts, but his samples proved barren.

The significance of the Kakahu conodonts lies chiefly in the implications stemming from their Upper Carboniferous age. As the first diagnostic Carboniferous fossils found in New Zealand they fill a gap in the country's fossil record which has been considered somewhat anomalous¹⁴. In the light of the broad regional setting of the Kakahu inlier the conodonts indicate that accumulation in the New Zealand Geosyncline began at least as early as the Pennsylvanian, thus supporting the suggestion of Fleming¹⁵, among others, regarding the time of initiation of sedimentation in this trough. Furthermore, the conditions of accumulation at least locally within or adjacent to the trough were suitable for the deposition of unusually pure limestones totalling some hundreds of feet in thickness. Conversely, the conodonts disprove the Lower Palaeozoic age of the Kakahu rocks and thus also the suggestion that rocks of this age outcrop east of the median tectonic line.

* Range extended by recent work of Scott and Collinson³ and Bender and Stoppel⁴.

Survival of determinable conodonts in the chlorite 2 subzone of the Kakahu inlier points to the possibility of their presence in rocks of similar metamorphic grade elsewhere; this observation could prove significant in establishing the age of parts of the Torlesse and Haast Schist Groups.

The Kakahu conodonts are the most southerly occurrence of these problematical fossils known to us. The nearest known Pennsylvanian conodonts are from south-eastern Queensland, Australia¹⁶.

We thank G. Mansergh of the NZ Geological Survey for information regarding the Kakahu limestones and for supplying the sample which first yielded conodonts, and M. Reid, Geology Department, University of Canterbury, for processing it. Mr D. R. Gregg kindly assisted with relevant literature. The research was supported by a University of Canterbury Grants Committee research grant.

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BIOLOGICAL SCIENCES

Functional Organization of Genetic Material as a Product of Molecular Evolution

MOLECULAR evolution consists of a sequence of events in which originally rare molecular mutants (DNA changes) spread into the species. Two important classes of mutations are nucleotide (or amino-acid) replacement and gene duplication.

From comparative studies of the protein sequences of related organisms, we now have some information on the rate of amino-acid substitution in evolution. The rate is different from protein to protein, but for each particular molecule, such as haemoglobin α or cytochrome c, it is remarkably uniform per year in diverse lines over geological times¹. This constitutes very strong evidence for the hypothesis¹⁻³ that most nucleotide substitutions in evolution are the result of random fixation of selectively neutral or near neutral mutants. Furthermore, the rate at which neutral mutant genes are substituted in the

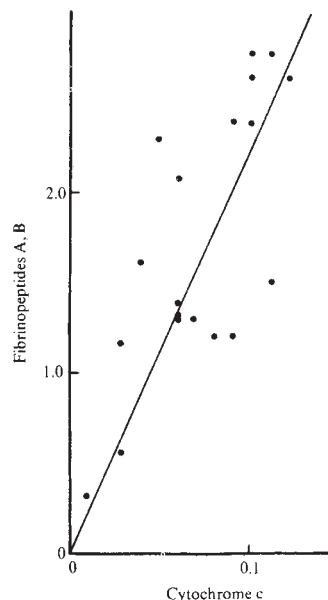


Fig. 1 The regression of the rate of amino-acid substitution of fibrinopeptides (actually their rapidly evolving parts) on that of cytochrome c. In each coordinate, the number of different amino-acid sites between two homologous proteins is expressed in terms of $-\log_e(1-p_d)$ where p_d is the fraction of differing sites. Dots represent twenty-one observed values (of which two sets of values coincide) from paired comparisons involving seven organisms (human, rhesus monkey, rabbit, dog, horse, pig and kangaroo). The estimated regression coefficient is 22.4.

population per generation is equal to the rate of occurrence of such mutants per gamete⁵.

There are significant differences in evolutionary rates among proteins. The highest rate (fibrinopeptides) is some 1,500 times greater than the lowest rate (histones)⁴. This means that the rate depends on the functional requirement of the molecule (refs. 1 and 3 and unpublished work of M. K. and T. O.). The greater the chance that the mutations are deleterious, the lower the evolutionary rate. The uniformity of evolutionary rate for each molecule implies that the fraction of neutral mutations among all mutations in each cistron remains constant per year.

The average rate of amino-acid substitutions estimated using nine proteins is about 1.6×10^{-9} /amino-acid site/yr³. If the difference of evolutionary rates among proteins is due to the difference in functional requirement rather than the difference in their mutation rate, we should expect that for any cistron the frequency of mutation at the time of occurrence is equal to that of the fibrinopeptides showing the highest substitution rate. This allows us to estimate the true mutation rate at the molecular level.

From the atlas of Dayhoff⁶ we sampled seven organisms for which the complete amino-acid sequences of fibrinopeptides A, B and cytochrome c are given, from which to calculate the regression of substitution rate of the fibrinopeptides on that of cytochrome c for which the rate of substitution is well known. We therefore chose the rapidly evolving parts corresponding to the amino-acid positions four to seven in fibrinopeptide A and nine to twenty in fibrinopeptide B (alignment 9 of Dayhoff⁶). The remaining amino-acid positions not only evolve much more slowly but also show some non-randomness with respect to the pattern of substitution. We have therefore excluded these slowly evolving parts from our calculation. Fig. 1 illustrates the regression based on all the pairs of comparisons between seven organisms (human, rhesus monkey, rabbit, dog, horse, pig and kangaroo) with observed values shown by dots. The abscissa gives the number of substitutions in terms of $-\log(1-p_d)$ for cytochrome c while the ordinate gives that for fibrinopeptides, where p_d is the fraction of different amino-acids. (For the rationale of using such a logarithmic

scale, see ref. 1.) The regression is about 22.4, suggesting that the true mutation rate is 22.4 times the substitution rate of cytochrome c, which is about 0.37×10^{-9} /amino-acid site/yr in mammalian evolution. The resulting mutation rate becomes

$$0.37 \times 10^{-9} \times 22.4 \approx 8.3 \times 10^{-9}$$

per amino-acid site/yr. This is roughly five times the average rate of substitutions of the nine proteins and agrees with the conclusion of Corbin and Uzzell⁷, and with the conventional mutation rate per locus per generation of $10^{-5} \sim 10^{-6}$ if the average cistron codes for the protein of several hundred amino-acids long and if the average generation time is not much different from 1 yr.

If a large fraction of mutations are deleterious as discussed above, every higher organism must suffer a heavy genetic load. According to the Haldane-Muller principle⁵ the mutation load of equilibrium populations is equal to one to two times the total deleterious mutation rate depending on the degree of dominance of mutations⁸. Recent investigations in *Drosophila* have shown that the detrimental mutations have a fairly high degree of dominance^{9,10}. The mutation load must therefore be nearly twice the total mutation rate, although epistatic (synergistic) interaction in fitness among the loci may reduce the load somewhat^{10,11}.

Various species of mammals have about the same amount of DNA. The human haploid genome consists of about 3×10^9 nucleotide sites^{12,13}, equivalent to 10^9 amino-acid sites: thus the total mutation rate due to base replacement per genome per year amounts to

$$8.3 \times 10^{-9} \times 10^9 \approx 8$$

If the mutation rate is strictly proportional to chronological time, the rate per generation is probably twenty times this figure in man but half as large in mouse. In the main course of mammalian evolution, the average generation time was probably 2~3 yr, so we tentatively equate 1 yr with one generation in mammals. If most of these mutations are deleterious, any mammalian species must suffer an intolerable mutation load: Muller argued¹⁴ that the total rate for detrimental mutations in man is at most 0.5 per generation. What does such great discrepancy signify? We conclude that a large part of DNA in the genome is not essential for the life of the organisms in that base substitutions have no effects³. If we compare the above estimate of the total mutation rate with Muller's maximum detrimental mutation rate we are led to the conclusion that only 0.5/8 or 6% of the total DNA are as important in function as the cistrons such as those coding for cytochrome c or haemoglobins. If the estimated total mutation rate per generation is twenty times that per year and the total detrimental mutation rate is 1.0 (taking into account the "viability polygenes" of Mukai¹⁵), then the fraction becomes 0.6%. Although we conclude, with Muller¹⁶, that the total number of "genes" in man is about 3×10^4 , whereas he assumes a large gene size (corresponding to 30,000 amino-acids), we assume that the genes are much smaller in size (corresponding to several hundred amino-acids on average) but a large fraction of DNA is not "informational".

Mammalian DNA is about 1,000 times larger than bacterial DNA, so that the genetic material duplicated some ten times on average ($2^{10} = 1,024$) in the course of evolution from a unicellular organism to the mammals¹⁷. After each fixation of duplication in the species, irrespective of whether the duplicated part is a whole chromosome or a tiny fraction, the duplicated genes must have differentiated from the original genes through nucleotide substitution. It is possible that in this process many mutations which would have been deleterious before duplication become harmless (selectively neutral) if they occurred after duplication, for one set of genes provides the essential function of the organism. The originally deleterious mutants will spread into the population by random drift, so that there will be degeneration in many duplicated genes. The possibility of DNA degeneration after gene duplication has

been pointed out by Ohno *et al.*¹⁷ and Nei¹⁵. The probability of such degenerated parts acquiring new functions might be quite small. Some duplicated genes will acquire new functions, establishing themselves as important genes of the organism. But mutation is a random event, so that the chance that duplicated genes will acquire a new function must be much smaller than that they will become inert. Thus, we should expect that higher organisms have much non-informational (inert) DNA in their genome.

In this case, the rate of nucleotide substitution must be very rapid within those parts, for all the mutations are neutral. We estimate this rate approximately as $1/3 \times 8.3 \times 10^{-9}$ /yr/nucleotide site, which is one-third of the estimated rate per amino-acid sites in rapidly evolving portions of the fibrinopeptides. We further multiply this by 1.2 to give total nucleotide substitution rate by taking account of the synonymous mutations¹⁹. The resulting rate is 0.33×10^{-8} /yr/nucleotide site. This is about five times as high as the rate estimated from the average proteins.

Recent investigations using DNA hybridization techniques indicate that the differentiation of DNA in mammalian evolution is very rapid. Laird *et al.*²⁰ estimated that the rate of DNA evolution is about three times as fast in artiodactyls and thirty times in rodents as the corresponding rate inferred from haemoglobins. Walker²¹ estimated that the differentiation of DNA of rodents is fifteen times as fast as that of known proteins. It is possible that the evolutionary rate of total DNA is more nearly proportional to generations rather than to chronological time. More recently, Kohn²², who has summarized these results, emphasizes that the substitution rate in DNA of mammalian genome is proportional to generations. He concludes that the number of nucleotide substitutions in non-repeated DNA is roughly five per genome per generation, and the total rate of substitutions is probably two to four times greater. It is interesting that his figure is roughly equal to our estimate, that is, eight substitutions per genome per year.

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Toxicity of the Photoisomers of Cyclodiene Insecticides to Freshwater Animals

SUNLIGHT and ultraviolet light convert aldrin, dieldrin and heptachlor to their corresponding isomers, photoaldrin, photodieldrin and photoheptachlor respectively, which are more toxic to mosquito larvae and housefly adults¹⁻³. In these insects the onset of toxic symptoms is much faster with the photoisomers than the parent compounds and has been assumed to be due to their rapid oxidative dehydrochlorination to lipophilic ketones^{3,4}. The effect of photoisomerization of isodrin is opposite to that seen with other photoisomers. Photoisodrin, because of its rapid detoxification, is far less toxic than isodrin to these two insects^{4,5}.

Table 1 Toxicity of Cyclodienes and their Photoisomers to Some Freshwater Animals

Insecticide	LD ₅₀ (p.p.m.)*			
	Bluegills	Minnows	<i>Asillus</i>	Mosquitoes †
Aldrin	0.26	—	0.08	0.003
Photoaldrin	0.09	—	0.04	0.0005
Dieldrin	0.17	0.024	—	0.006
Photodieldrin	0.03	0.010	—	0.003
Heptachlor	—	0.013	0.10	0.005
Photoheptachlor	—	0.008	0.06	0.002
Isodrin	0.012	—	—	0.019
Photoisodrin	0.025	—	—	0.058
Endrin	0.010	—	—	0.017

* Mortalities recorded after 24 h.

† Data from refs. 1-3 for *Aedes aegypti* larvae.

The toxicity of all these photoisomers to freshwater animals which constitute food chains has not been reported. Because cyclodiene insecticides have been used extensively, the hazards of their persisting residues may be magnified by concentration of the lipophilic metabolites of their photoconversion products in food chains (Georgacakis and Khan, unpublished work). We report here the results of a preliminary investigation on the toxicity of these photoisomers to some freshwater animals.

We used animals collected from natural conditions or purchased from local suppliers. The fish were acclimated at

65° F in an environmental room for at least 5 days in mixtures of habitat water and distilled water in the following respective proportions: 2 : 1, 1 : 2, 1 : 8, and distilled water only. The distilled water was aged at this temperature for about 2 weeks with constant aeration (oxygen concentration 10 p.p.m.). Fish were exposed in 1 pint mason jars containing 200 ml. of constantly aerated water. Each jar contained no more than four fish, tadpoles or crayfish and about five replicates (twenty animals) were used for each concentration. Microcrustaceans were exposed in 25 ml. of water in plastic Petri dishes (five to ten animals per dish) with at least two replicates. Each experiment was repeated at least twice and the average values of the two used to determine LC₅₀ or LT₅₀ values⁴. Assays were performed at 65° F using the distilled water in which the fish were acclimated. Six graded concentrations (logarithmic dosages) between predetermined dosages which caused zero and 100% mortality were used to determine LC₅₀ values.

Table 1 shows LC₅₀ values of the cyclodienes and their photoisomers to fish, crustacea and insect. To the fish isodrin and endrin are the most toxic insecticides. Photoisodrin is about two times less toxic than isodrin to all these animals. Photoaldrin, photodieldrin and photoheptachlor are more toxic than their parent compounds aldrin, dieldrin and heptachlor, respectively. Photoaldrin seems to be specially toxic to mosquito larvae¹. The onset of toxic symptoms leading to death is much faster with photoaldrin, photodieldrin and photoheptachlor than their parent compounds as judged by LT₅₀ values for a number of aquatic animals (Table 2). Again, photoisodrin seems to be less toxic than isodrin or endrin; tadpoles, fish, isopods and *Daphnia* are more sensitive than *Gammarus*, crayfish and planarians. Toxicity of photodieldrin has been reported for only a few animals⁶⁻⁸, where it has been found to be two to four times more toxic to rats, mice, guinea-pigs and pigeons, equally toxic to beagles and less toxic than dieldrin to the domestic fowl. To the freshwater animals tested here, however, photoaldrin, photodieldrin and photoheptachlor are more toxic than their parent compounds. Photoisodrin is less toxic than isodrin or endrin. Because the first three photoisomers can be converted to more toxic lipophilic ketones^{3,4}, the toxicity of these photoisomers produced by sunlight^{1-3,8} or by microorganisms⁹ may be magnified by the biological concentration¹⁰ of lipophilic ketones in animal food chains. The possible hazards of such a magnification are obvious, and research on the analysis of these photoisomers and their ketones in the environment, especially in the Great Lakes region, is badly needed.

Table 2 Toxicity of Cyclodienes and their Photoisomers to Various Freshwater Animals

Animal *	LT ₅₀ : min of continuous exposure					Photoisodrin
	Aldrin	Photoaldrin	Dieldrin	Photodieldrin	Endrin	
Crustacea						
Freshwater flea, <i>Daphnia pulex</i> (1)	290	205	280	220	180	380
Freshwater isopod, <i>Asillus</i> spp (1)	160	75	80	50	35	55
Freshwater amphipod, <i>Gammarus</i> spp (1)	2,200	1,200	2,200	180	150	350
Freshwater crayfish, <i>Cambarus</i> spp (1.8)	1,140	390	1,000	200	200	1,000
Planaria						
Freshwater flatworm <i>Dugensia</i> spp (1)	—	—	400	300	300	800
Amphibia						
Tadpoles (1)	—	—	50	30	—	—
Fish						
Guppies, <i>Gambia affinis</i> (1)	—	—	90	35	—	130
Minnows, <i>Pimephalus promelas</i> (0.7)	—	70	100	90	55	130
Bluegills, <i>Lepomis macrochirus</i>	> 360 †	100 †	> 360 †	70 †	360 ‡	440 ‡

* Values in parentheses show the concentration to which the animals were exposed.

† Concentration was 0.24 p.p.m.

‡ Concentration was 0.2 p.p.m.

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Drug Dependence and Phenotypic Masking in *E. coli*

STREPTOMYCIN dependent (Sm^D) strains of *E. coli* L1 (which has an amber mutation in the ornithine transcarbamylase gene which is streptomycin suppressible) will grow when subcultured on Sm agar (500 μ g/ml.) or on 3% EtOH agar, but not on paromomycin agar (Pm) or kanamycin (Km) agar, or any mixture of two of the drugs named (Table 1)¹. Paromomycin dependent (Pm^D) mutants selected on Pm agar (200 μ g/ml.), however, would grow on subculture to Pm or EtOH agar, EtOH + Pm, EtOH + Km or Pm + Km drug agars; but would not grow on Sm or Km agar, or on any other drug agar which contained streptomycin. These obvious phenotypic differences between Sm^D and Pm^D mutants were eliminated if the strains were first grown on EtOH agar and then subcultured to the different drug agars, when both types of dependent mutant were found to grow on EtOH or Sm or Pm agar, or on EtOH + Pm or Pm + Km agar (Table 1). Gorini *et al.*¹ considered the latter to be the true phenotype of the strains (which after growth on EtOH agar were called Drug^D) which had previously been masked by Sm or Pm which had so altered the ribosomes as to mask the true properties of the cells. They called the phenomenon "phenotypic masking".

We have investigated whether strains of *E. coli* other than L1 also exhibited phenotypic masking, and if so whether the same phenotypic pattern was eventually expressed by Sm^D and Pm^D mutants of different origin. Further, because ethanol and the aminoglycosides have no chemical similarity, we were particularly interested to test whether "the mutants are equally dependent either on ethanol or streptomycin or paromomycin . . .".¹

Initial experiments were performed with *E. coli* SD, a streptomycin dependent strain, and *E. coli* BQPD, a paromomycin dependent strain selected by plating a sensitive strain (*E. coli* BQ) on 200 μ g paromomycin sulphate/ml. nutrient agar. When these strains were plated on various drug agar plates, as Gorini *et al.*¹ had done, we obtained the growth pattern given in Table 1. Unlike that of the Harvard group, our streptomycin dependent strain grew on Sm + EtOH agar,

as did both the streptomycin and paromomycin dependent strains after conversion to Drug^D strains by growth in alcohol agar. Our result for the Sm^D strain SD in regard to Sm + EtOH seems quite consistent with the behaviour of both our and Gorini's Pm^D strains which grow on Pm + EtOH agar. Again, although derived from different parent strains, after "unmasking" both SD and PD give growth on Sm + EtOH, whereas the Gorini Drug^D strains did not. Our Pm^D strain also differed in not growing on Km + EtOH.

Table 1 Phenotypic Characterization of Streptomycin and Paromomycin Dependent Strains of *E. coli*

Drug in plating medium	Drug provided for growth before plating					
	Streptomycin strain *L1 Sm^D	SD	Paromomycin strain *L1 Pm^D	PD	Ethanol Drug ^D strain *(L1 Sm^D or Pm^D)	(SD or PD)
None	±	±	±	±	0	0
EtOH	+	+	+	+	+	+
Sm	+	+	0	0	+	+
Sm + EtOH	0	+	0	0	0	+
Sm + Pm	0	0	0	0	0	0
Sm + Km	0	0	0	0	0	0
Pm	0	0	+	+	+	+
Pm + EtOH	0	0	+	+	+	+
Pm + Km	0	0	+	+	+	+
Km	0	0	0	0	0	0
Km + EtOH	0	0	+	0	0	0

Concentrations of drugs whenever used: ethanol, 3%; Sm, 500 μ g/ml.; Pm, 200 μ g/ml.; Km, 100 μ g/ml.

+, Growth after 24 h; 0, no growth even after 48 h; ±, some growth after 48 h, incubation at 37° C.

* The first column of each pair records the results of Gorini *et al.*¹.

When strains are transplanted directly from Sm media to Pm media or vice versa, no growth is obtained, nor can Drug^D strains of either derivation be grown on mixtures of Sm and Pm, which are always lethal.

To study the ability of the different drugs to support growth, we assumed that our Drug^D strains were of the same genotype (which seems likely, for they exhibited identical phenotype to these drugs irrespective of origin) and evaluated the viability of Drug^D SD and Drug^D PD on various drug agars. For these experiments the strains were first grown on 3% EtOH agar for 48 h, colonies were transferred to 3% EtOH nutrient broth and further incubated at 37° C for 21 h to give liquid cultures of Drug^D SD and Drug^D PD. Ten-fold dilution series were made in nutrient broth and 0.2 ml. spread/plate (duplicate) on each of the various drug agars listed in Table 2, where the results are given.

The parent cultures were grown in EtOH broth from small inocula, so it was assumed that the maximum survival would be obtained on EtOH agar and these counts were taken to represent the true viabilities of the cultures (100% survival). For these strains Sm agar is nearly as good a recovery medium as is EtOH agar (Table 2). On the other hand, Pm agar or mixtures of Pm + EtOH or Pm + Km agar and Sm + EtOH agar supported the survival and outgrowth of about 10⁻⁴ to 10⁻⁵ less cells, while all the other drug agars tried did not permit growth of the Drug^D strains.

It seems clear from this and similar experiments with other drug dependent strains of *Escherichia*, *Staphylococcus*, *Proteus* and *Bacillus*, to be reported, that Drug^D mutants do not form "a single class in which their dependence is equally satisfied by several drugs"¹. Differences were also obvious in colony size and morphology: the typical coliform colony was obtained on Sm agar but atypical (small and "frosted") colonies were obtained on Pm and EtOH agar.

We have confirmed the finding that Sm/Pm mixtures are only lethal if both drugs are present in concentrations at, or above, the minimum concentrations required to kill the parent

sensitive strain. It seems from this that the minimum inhibitory concentration of an aminoglycoside antibiotic is the external concentration of drug required to "saturate" the internal ribosome complement of the particular strain in the prevailing environmental conditions, cation concentration in particular. Assuming that "saturation" is brought about by drug molecules actually attaching to specific sites on the ribosomes²⁻⁴, then the addition of a second aminoglycoside would lead to "double saturation" and "double kill" in the case of a sensitive strain. A strain resistant to one, but not the other, would still be killed by the drug saturating the ribosomes (assuming that resistance in this case is by non-attachment, the specific site being unavailable).

Table 2 Survival of Drug^D Dependent Mutants (after Growth in 3% Ethanol) on Transfer to Various Drug Agar Media

Drug in plating medium	Survival (%) (taking survival on EtOH agar as 100%)	
	Drug ^D SD	Drug ^D PD
None	0*	0*
EtOH	100	100
Sm	80	73
Sm + EtOH	0.03	0.004
Pm	0.02	0.004
Pm + EtOH	0.03	0.002
Pm + Km	0.02	0.004

No survivors were obtained on any of the following drug agars: Sm+Pm; Sm+Km; Sm+Nm; Pm+Nm; Km; Km+EtOH; Km+Nm; Nm; Nm+EtOH.

The strains SD and PD were grown in 3% ethanol broth at 37° C for 21 h before a decimal dilution series of each was made (in drug-free broth) to 10⁻⁶. Aliquots (0.2 ml.) of each dilution from 10⁻¹ to 10⁻⁶ were plated in duplicate on each type of agar or drug agar listed (total 192 plates per strain) and colonies counted after 48 h of growth at 37° C. The concentrations of drugs in the plates were: ethanol, 3%; Sm, 100 µg/ml.; Pm, 50 µg/ml.; Km, 100 µg/ml.; Nm, 50 µg/ml.

* Some very tiny colonies were visible after 48 h of incubation.

Why then should Sm/Pm mixtures kill the dependent strains which can grow (indeed depend for their growth) on either streptomycin or paromomycin? It seems likely that there are two separate sites on the ribosome for the attachment of these drugs, for if there were a single site there would be straightforward competition for attachment and whichever became attached growth would result. It is now well known that Sm attaches to the P₁₀ protein of the 30S subunit⁵. We suggest that Pm also attaches to the P₁₀ protein close to the Sm site so that, when both Pm and Sm are attached, the complex so formed prevents either drug from producing the message-correction required for survival of dependent strains, e.g. suppression of the amber mutation in the OTC message of the conditionally Sm dependent strain of Gorini and Kataja⁶. This hypothesis would also explain why Gorini, Rosset and Zimmermann¹ found "by transduction mediated by bacteriophage P1 . . . that the entire complex phenotype described above is transferred in one step from a Drug^D donor to a wild type recipient on selecting for resistance to any one of the drugs to which the donor is resistant or dependent".

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Cell Division after Laser Microirradiation of Mitotic Chromosomes

MITOTIC chromosomes are now being studied by laser microirradiation which involves removing small areas (0.5–2 µm long) of either DNA or protein¹. The cells are then observed in tissue culture or fixed and stained cytochemically. By these means, it has been possible to examine nucleolus production and function by selectively altering the nucleolar organizer region of chromosomes². This type of investigation requires only that the cell completes mitosis and survives in interphase long enough for nucleolus formation and function to be assayed. Although much information can be gained from this type of short term experiment, there would be more scope for investigation if irradiated cells could be cultivated for long periods of time, and shown to be capable of undergoing subsequent mitoses.

Until recently it was only possible to surmise that irradiated cells could undergo additional mitoses. In previous studies of chromosome microirradiation we used primary cultures of salamander lung epithelium. These cells remain flat during mitosis permitting precise microirradiation of desired chromosome regions. By treating the cells with acridine orange solution (1–0.01 µg/ml.) for 5 min it was possible to sensitize the chromosomal DNA to intense visible laser light³. The cells remained viable, reforming nucleoli and nuclear membranes. We have observed many cells for as long as 3 weeks in culture, and two reached the next metaphase, although neither of them completed division. This type of investigation was particularly difficult because the time between mitosis in these cells may be several weeks. In addition, since it is a primary culture, many cells will not even go through another division.

Because of the inherent difficulties of working with primary cultures of a cell type with an erratic mitotic rate, we looked for cells with more desirable features. We wanted cells with a rapid mitotic rate, which remained flat during mitosis so that the chromosomes were continually visible, and which had relatively few large chromosomes. We found these features in an established line of cells of the rat kangaroo (*Potorous tridactylis*)⁴, which have eleven chromosomes, varying from 1 to 10 µm long, a mitotic doubling time of 24 h and, like many marsupial cells, do not round up during mitosis.

We have investigated whether these cells could divide after laser-irradiation of the chromosomes. The procedures of microirradiation were similar to those described before^{2,3}, except that 0.001 µg/ml. of acridine orange was used instead of 0.01 µg/ml. Rat kangaroo cells seemed to respond to a slightly lower dye plus laser treatment than do the salamander cells. After two pulses of the laser with a cumulative imposed energy in the focal spot of 84 µJ, chromosomal alterations similar to those described before were produced¹⁻³. The anaphase cell in Fig. 1 had been irradiated in this manner, followed by time lapse cinematography. One chromosome of each of the two groups was irradiated (the arrow indicates the lesion in one anaphase group). The additional photographs in this figure are abstracts from time lapse footage of both of the cells formed from the above anaphase cell. Twenty-six hours after irradiation, both cells underwent normal mitosis, apparently passing through G₁, S, and G₂, for chromosome duplication, nucleolus disappearance and spindle formation, all occurred in the usual time.

This experiment should dispel doubts that cells with microbeam irradiated chromosomes can continue to divide. It

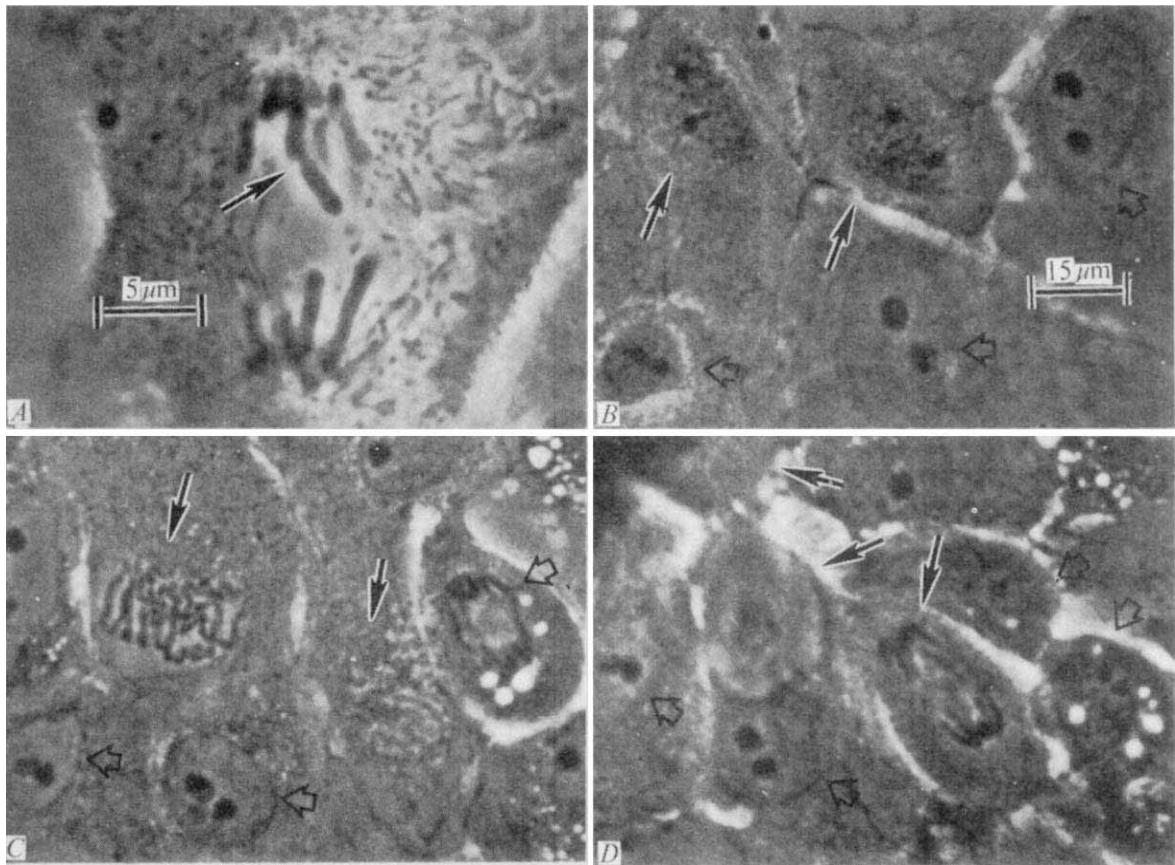


Fig. 1 Mitosis 26 h after laser microirradiation of chromosomes. *A*, Chromosome arm immediately after irradiation; the arrow indicates the altered region. *B*, 1 h after irradiation; closed arrows indicate the two daughter cells, open arrows indicate cells adjacent to irradiated cells. *C*, 25 h after irradiation; the two irradiated cells are indicated by closed arrows, one is in the middle prophase, and the other is in early prophase, one adjacent cell (open arrow) is in late anaphase. *D*, 26 h after irradiation; one of the irradiated cells has completed mitosis (closed arrows point to the two cells formed from this division), and the other irradiated cell is in late anaphase (closed arrow). Illustrations *B*, *C* and *D* are abstracts from a 16 mm time lapse film.

certainly seems feasible to establish clones from cells irradiated in this fashion. Yet the fate of the altered DNA segment of the chromosome remains to be established. It seems unlikely that the normal nucleotide sequences could be re-established in such a large segment of altered DNA.

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Single-gene Heterosis in *Drosophila* revealed by Inbreeding

KIMURA and others¹⁻⁴ have suggested that most observed isoallelic variation in natural populations is maintained by mutation pressure and drift alone, and that selection is not

involved. This viewpoint has been criticized^{5,6}, and several models⁷⁻¹¹ have been proposed which permit large amounts of selectively maintained polymorphism without impossibly high genetic loads. The controversy between proponents of selection and those favouring neutrality is far from resolved, and it has stimulated a great deal of work. We report here a case of conditional heterosis in *Drosophila pseudoobscura* which we feel can reasonably be attributed to the polymorphic locus being examined.

One consequence of a model proposed by Milkman⁹ and elaborated by Wills, Crenshaw and Vitale¹¹ is that if there are many polymorphisms maintained by heterotic selection in a population, the selection coefficients associated with each of these polymorphisms will be very small. If most of the genes in the population are monomorphic, however, the selection coefficients associated with the remaining polymorphisms will be much greater. This is because the relative, rather than the absolute, heterozygosity of each organism would determine its relative fitness in the population. We reasoned that inbreeding might reveal selection coefficients associated with loci which seemed to be neutral in outbred populations.

We used a highly polymorphic population of *Drosophila pseudoobscura*, obtained in 1964 by C. W. in Strawberry Canyon, Berkeley, California, and maintained since by Dr S. Prakash. A large number of single pair matings were set up using these outbred flies, and several pure lines were obtained for each of the following electrophoretically distinguishable isoalleles: octanol dehydrogenase (ODH, autosomal, not chromosome III) 1.00 and 1.22, and esterase-5 (E-5, sex-linked) 1.00 and 1.21 (refs. 12 and 14). Both these enzymes are dimeric, and form a hybrid band in heterozygotes. The pure lines of each allelic

type were pooled and maintained subsequently in mass culture to form the P_1 . Single pair matings between ODH 1.00/1.00 and 1.22/1.22 gave flies all of which had the genotype 1.00/1.22. Brother-sister single pair matings were made from these cultures in turn to give the first inbred generation (I_1). Brother-sister single-pair matings were then made from the progeny of these crosses. When larvae appeared in the vials the parents were removed and their genotypes were determined (E-C vertical acrylamide gel electrophoresis). In one quarter of the vials both parents were heterozygous 1.00/1.22. These vials were retained and the remainder discarded. Flies emerging from them formed the I_2 generation, and these in turn were brother-sister mated. Cultures in which both I_2 parents were heterozygous were retained to give the I_3 , and this process was continued.

one 1.00/1.12 heterozygote to one homozygote for the gene carried by the male parent.

It has been demonstrated repeatedly that unusual or stress media emphasize genetic differences^{15,16}. Consequently, parents of the genotypes listed in the previous paragraph were allowed to lay eggs on corn meal-molasses-agar medium to which either 0.25 M KCl or 3×10^{-3} M 1-octanol had been added. Egg-to-adult survival on both these media was poor.

The numbers of progeny flies of each genotype at the P_1 and I_{12} levels of inbreeding surviving on both KCl and octanol stress media are listed in Table 1. In most cases there was no significant deviation from Mendelian expectation. There was, however, a highly significant deviation in I_{12} ODH males surviving on the octanol medium. An excess of heterozygotes and deficiency of 1.00/1.00 homozygotes was observed in

Table 1 Genotypes and Numbers of Outbred and Inbred *Drosophila pseudoobscura* surviving on Stress Media

Medium	Inbreeding level		Genotype*			χ^2	P	
(a) Flies segregating for ODH								
0.25 M KCl	P ₁		1.00/1.00	1.00/1.22	1.22/1.22			
		♂♂	29 (27)	57 (54)	22 (27)	1.24	0.75 > P > 0.5	
		♀♀	33 (27.25)	52 (54.5)	24 (27.25)	1.72	0.5 > P > 0.25	
	I ₁₂	Line A	♂♂	19 (18)	40 (36)	13 (18)	1.89	0.5 > P > 0.25
			♀♀	22 (24)	55 (48)	19 (24)	2.23	0.5 > P > 0.25
		Line B	♂♂	26 (23.5)	43 (47)	25 (23.5)	0.70	0.75 > P > 0.5
			♀♀	29 (26)	55 (52)	20 (26)	1.90	0.5 > P > 0.25
		Line C	♂♂	22 (22)	46 (44)	20 (22)	0.27	0.9 > P > 0.75
			♀♀	25 (22.75)	42 (45.5)	24 (22.75)	0.56	0.95 > P > 0.9
	I ₁₂	Pooled	♂♂	67 (63.5)	129 (127)	58 (63.5)	0.70	0.75 > P > 0.5
I ₁₂	Pooled	♀♀	76 (72.75)	152 (145.5)	63 (72.75)	1.74	0.5 > P > 0.25	
3 × 10 ⁻³ M 1-octanol	P ₁		♂♂	30 (33.25)	68 (66.5)	35 (33.25)	0.44	0.9 > P > 0.75
			♀♀	39 (32.75)	63 (65.5)	29 (32.75)	1.72	0.5 > P > 0.25
	I ₁₂	Line A	♂♂	9 (18)	44 (36)	19 (18)	6.33	< 0.05
			♀♀	20 (18)	37 (36)	15 (18)	0.75	0.75 > P > 0.5
	I ₁₂	Line B	♂♂	16 (22.5)	58 (45)	16 (22.5)	7.51	< 0.025
			♀♀	16 (23.75)	59 (47.5)	20 (23.75)	5.91	0.05
		Line C	♂♂	13 (24)	59 (48)	24 (24)	7.56	< 0.025
			♀♀	16 (18.75)	33 (37.5)	26 (18.75)	3.75	0.25 > P > 0.1
	I ₁₂	Pooled	♂♂	38 (64.5)	161 (129)	59 (64.5)	19.29	< 0.001
	I ₁₂	Pooled	♀♀	52 (60.5)	129 (121)	61 (60.5)	1.73	0.5 > P > 0.25
(b) Flies segregating for E-5								
			Homozygotes	Heterozygotes				
0.25 M KCl	I ₁₂	♀♀	143 (142)	141 (142)	0.01	0.995 > P > 0.99		
3 × 10 ⁻³ M 1-octanol	I ₁₂	♀♀	109 (120)	131 (120)	2.02	0.25 > P > 0.1		

* Expected numbers are given in parentheses.

Inbreeding of the E-5 lines was carried out in a similar way, except that because the males were hemizygous brother-sister matings were retained in which the female was heterozygous 1.00/1.12 and the male was either 1.00 or 1.12. This process of inbreeding, then, gradually made more and more of the genome homozygous except for the chromosomal regions immediately surrounding the ODH or E-5 loci. Several separate inbred lines were carried for both ODH and E-5. While there was considerable lethality and infertility in the first generations of inbreeding, by the I_8 this had disappeared. The fertility of the inbred flies, as measured roughly by the proportion of successful single-pair matings, rose by the I_8 to the outbred level of 80–90%.

Pure lines were obtained from the I_{12} and maintained in mass culture. Matings were carried out between P_1 and also between I_{12} pure lines to give flies of the following genotypes: P_1 ODH 1.00/1.22; I_{12} ODH 1.00/1.22; P_1 E-5 1.00 $\delta\delta$ and 1.00/1.12 $\eta\eta$; P_1 E-5 1.12 $\delta\delta$ and 1.00/1.22 $\eta\eta$; I_{12} E-5 1.00 $\delta\delta$ and 1.00/1.12 $\eta\eta$; I_{12} $\delta\delta$ and 1.00/1.12 $\eta\eta$. Flies of each of these types were allowed to mate *inter se*. The progeny of the ODH crosses would be expected to segregate in a 1:2:1 ratio. The females of the E-5 crosses should segregate in a ratio of

three different inbred lines of I_{12} ODH males, the pooled data yielding a χ^2 of 19.3, significant at the 0.001 level. A barely significant deviation similar to that seen in the males was observed in the females of one of the three lines.

Can this heterosis be attributed to the ODH locus itself? We believe it can. No deviation from Mendelian expectation was observed for the P_1 ODH flies on either stress medium. No deviation was observed for the I_{12} ODH flies on KCl medium, even though the genotype of their parents was identical to that of the parents of the flies raised on the octanol medium—and in most cases the parents themselves were identical, having been transferred from one stress medium to the other to continue egg-laying. The heterotic effect was thus confined specifically to the medium containing octanol, a substrate of ODH. That the octanol medium might be the cause of a generalized heterotic effect is rendered unlikely by the observation that I_{12} E-5 flies on either medium showed no excess of heterozygotes. A classical explanation for heterosis, that it is the consequence of masked subvital genes in the heterozygote, cannot be used here since survival of flies carrying all three ODH genotypes was normal on KCl medium.

The most likely explanation for the appearance of heterosis on inbreeding is that polymorphic modifiers of the ODH locus, perhaps other genes in the pathway of octanol metabolism, have been made homozygous by inbreeding. The effects of the ODH isoalleles themselves then become on inbreeding a much more important factor in the survival of the flies. The heterotic effect is far more pronounced in males than in females. Only one of the three I_{12} ODH lines showed a significant excess of heterozygotes in the females. This suggests that even after twelve generations of inbreeding modifiers sufficient to mask the heterotic effect are still segregating in the X chromosomes of two of the lines. Further inbreeding should clarify this point.

The use of specific stress media to detect selectional differences in highly inbred flies will, we hope, prove to be a general method for examining whether other polymorphisms are selectively neutral. Selection coefficients, though not heterosis, associated with the alcohol dehydrogenase locus of *D. melanogaster* have been detected by Gibson using a medium containing ethanol¹³. It is important to emphasize that the observed heterosis is conditional; that is, it is dependent on the particular environment. In Strawberry Canyon the ODH 1.22 allele is at a frequency of 0.023¹⁴. Though we have yet to determine if long-chain alcohols other than octanol produce effects on flies segregating for the ODH locus similar to those described here, one might speculate that large amounts of long chain alcohols are formed by fermentation in only a small proportion of the food supplies on which the larvae habitually grow. Selection for heterozygotes would thus only occur in a small fraction of the broods.

There are some similarities between the conditional heterosis at the ODH locus and that detected in sickle cell anaemia heterozygotes in man¹⁷. In the latter case, however, the heterotic effect is large enough to be observed in normally outbred humans, and one homozygote is a semi-lethal. Homozygotes for either ODH allele survive perfectly well when there is no octanol in the medium, so that ODH polymorphism imposes a far smaller genetic load on the population than does sickle cell anaemia—a load which might fall effectively to zero under laboratory conditions. Polymorphisms of the ODH type could, if numerous, permit the concealment of large amounts of genetic variability without large genetic loads. Homozygosity for these genes would not confer any great disadvantage on the organism but heterozygosity for them would, under some conditions, confer a slight advantage. How large this class of polymorphisms is remains to be seen.

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Survival of Mouse Embryos after Freezing and Thawing

COMPARED with the successful storage of spermatozoa and other tissue cells at sub-zero temperatures, attempts to preserve frozen mammalian eggs have been disappointing (reviewed in refs. 1 and 2), although a few pregnancies have resulted from the transfer of previously frozen fertilized rabbit eggs³ and unfertilized mouse eggs⁴ to recipient foster mothers. I now wish to describe a technique for obtaining a high proportion of viable embryos after subjecting them to sub-zero temperatures.

Random bred female albino mice (CFLP strain, Carworth Europe) 6–8 weeks old were superovulated with gonadotrophins⁵. Eight-cell embryos were flushed from the oviducts and anterior portion of the uterine horns of mated females 68–72 h later and early blastocysts were flushed from the uterine horns a further 24–28 h later. Embryos were collected in Dulbecco's phosphate buffered salt solution⁶ supplemented with 0.33 mM sodium pyruvate, 5.56 mM glucose, 3 mg/ml. bovine serum albumin and 100 IU/ml. penicillin G (potassium salt). The medium had a pH of approximately 7.2 and an osmolarity of 287.6 ± 0.9 mosmols; it was similar in composition to that used for storage of mouse embryos at temperatures between 0° and 10° C⁷. Embryos were washed in several changes of medium (2 ml./wash) to remove debris.

Between twelve and twenty embryos were placed in 0.25 ml. of the medium in a 5 ml. test-tube and cooled to 0° C on crushed ice. Then 0.25 ml. of medium plus 15% polyvinylpyrrolidone (PVP), previously cooled to 0° C, was added and 3–5 min were allowed for the mixture to equilibrate. The final concentration of PVP (7.5%) was half that used to freeze mammalian blood cells⁸. After equilibration the test-tube was placed in a mixture of acetone and dry ice at –79° C for 5 min. The rate of cooling from 0° to –79° C was approximately 1° C/s. The test-tube was transferred to a vacuum flask of dry ice and left for 30 min before thawing.

To thaw, the test-tube was placed in a water bath at 37° C and 1 ml. of medium, also at 37° C, was added to reduce the concentration of PVP as the medium thawed. The rate of warming to 20° C was approximately 1.4° C/s. The contents of the test-tube were emptied into a solid embryological watch-glass and embryos that looked normal were collected and washed through several changes of medium as quickly as possible because exposure to concentrations of PVP greater than 1% for prolonged periods adversely affects further development (my unpublished observations). Embryos were then transferred to a modified Krebs–Ringer bicarbonate medium⁹ and cultured by the method described by Brinster¹⁰. Table 1 gives the combined results of four experiments which were not significantly different. Of 186 eight-cell ova initially frozen, 139 (74.7%) apparently normal embryos were recovered on thawing and ninety-six (69.1%) of these developed into blastocysts after 48 h in culture.

The combined data for two experiments with early blastocysts are given in Table 1, for again there was no significant difference. Fifty early blastocysts were frozen; thirty-four (68.0%), which were apparently normal, recovered, thirty-one (91.2%) of which expanded fully after culturing for a further 24 h at 37° C.

Although there was no significant difference between the number of normal looking eight-cell ova and early blastocysts recovered after freezing, the subsequent development of the early blastocysts in culture was better than the eight-cell ova ($\chi^2_{(1)} = 8.02$; $0.01 > P > 0.001$).

To test further the viability of the embryos after thawing, nineteen eight-cell ova were transferred after thawing to the ampullar region of the left oviducts (six to seven per oviduct) of three F₁ hybrid females (C57Bl × CBA) on day 1 of pseudopregnancy¹¹ (day 1 being the day on which the vaginal plug was found). On day 18 of pregnancy two females were pregnant; the left uterine horn of one had three normal looking albino foetuses and two resorptions from the six eight-cell ova transferred, the other female had one albino foetus and three resorptions out of seven eight-cell ova transferred. Thus nine out of nineteen (47.4%) implantations and four out of nineteen (21.1%) foetuses resulted from the transfer of eight-cell ova previously frozen and thawed.

Table 1 Development *in vitro* of Mouse Embryos previously Frozen and Held for 30 min at -79°C before Thawing at 37°C

Developmental stage	No. of experiments	No. of embryos frozen	No. of embryos recovered	No. of blastocysts after culture	Blastocysts (%)
Eight-cell	4	186	139	96*	69.1
Early blastocysts	2	50	34	31†	91.2

* Cultured for 48 h after thawing.

† Cultured for 24 h after thawing.

Experiments combined as they were not significantly different.

Thirteen expanded blastocysts, obtained after freezing and thawing the early blastocysts and culturing them for a further 24 h, were transferred to the left uterine horns of two F₁ hybrid females (C57Bl × CBA) on day 3 of pseudopregnancy (six and seven per horn respectively)¹². Six and three albino mice respectively were born to the two females. Thus nine out of thirteen (69.2%) blastocysts previously frozen developed into normal young after transfer. Again, viability after freezing and thawing was significantly greater than that of the eight-cell ova ($\chi^2_{(1)} = 8.46$; $0.01 > P > 0.001$).

These results demonstrate that mouse embryos can be frozen and thawed successfully if a suitable protective agent is added to the medium before freezing. Protective agents such as glycerol and dimethyl sulphoxide had previously been found to be toxic to embryos above 0°C ⁷ and I found that 'Dextran T70' (Pharmacia) and sucrose failed to protect the embryos during freezing. 'Ficoll' (Pharmacia) protected a few embryos but so far PVP has been the most effective protective agent. The blastocysts seemed to be more resistant to freezing than the eight-cell embryos, which is inconsistent with the observation¹³ that rabbit blastocysts were less resistant than earlier stages to storage at about 10°C .

With the perfection of this technique, the storage of mutant strains of mice not in current use but of potential interest could be possible. At the appropriate time the stored embryos could be transferred to foster mothers and the resulting offspring used to re-establish the mutant strain. The application of this technique to the large domestic animals could facilitate the dissemination of stock of good genetic background and possibly shorten the generation time for progeny testing. Low temperature storage combined with the sexing of embryos, when this method is developed, would have tremendous scope for the improvement of livestock in the future.

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Unsuccessful Attempts to Transfer Morphine Tolerance and Passive Avoidance by Brain Extracts

SEVERAL positive and negative reports have been reviewed recently^{1,2} on the possibility of transferring acquired behaviour from animal to animal by injecting brain extract from a trained donor into an untrained recipient. We were interested in the possibility of transferring morphine tolerance from rats to mice, as reported by Ungar and Galvan³. We failed to replicate this finding, as had other investigators^{4,5}. Nevertheless, we felt it worthwhile to search for the appropriate conditions and chose Ungar's simpler system of "fear transfer" as a model^{6,7}. Here rats are overtrained (by means of foot shock) to avoid entering a black box; recipient mice are then tested in the same box. To enable us to follow accurately the published procedures one of us (A. G.) spent a few days in Ungar's laboratory. In the next 3 months we carried out eighteen unsuccessful experiments with 125 donor rats and 383 recipient and saline control mice. We then did a blind test in our mice using control and trained donor extracts provided by Dr Ungar. Next, we sent 100 of our mice to Houston, for testing as recipients concurrently with the local strain. Finally, we selected, from all our experiments, those mice (of both sexes) which seemed to avoid the black box more often after receiving the extracts. These animals were bred and the offspring were tested as recipients. We hoped to select for recipient capability that might be under genetic influence. The results of all these experiments were negative or equivocal.

For tolerance the procedures of Ungar and Galvan³ were followed closely. Sprague-Dawley male rats (200–250 g) were made tolerant by three daily subcutaneous injections of morphine sulphate, starting with 10 mg/kg (in terms of the free base) and increasing to 100 mg/kg after 14 days. Rats were decapitated 12 h later, and brains were removed quickly and stored frozen on dry ice until required.

Recipients were thirty-four Swiss-Webster male mice (25–35 g), screened a week earlier as follows. First, they were tested for response on a 56°C hot plate⁸; all reacted within 30 s, after which morphine sulphate was given (35 mg/kg of free base intraperitoneally). After 30 min the mice were re-tested on the hot plate; two which reacted within 30 s were discarded. Morphine-induced running activity was also measured in special counter cages⁹. From the 4 h cumulative record, the sixteen most active runners were ranked, alternate mice being assigned to two groups. A coin was tossed to decide which group would receive physiological saline and which tolerant rat brain extracts. These solutions were administered intraperitoneally, and 24 h later mice were tested as in the screening procedure.

For passive avoidance procedures were as described by Ungar *et al.*^{6,7} except as noted. The apparatus for training and testing consisted of a white 'Lucite' box (30×30×15 cm high) with transparent cover, connected by a small black passageway to a black 'Lucite' box (25×10×15 cm high) with an opaque cover and a grid floor which could be electrified (80 V, 60 Hz). The apparatus was built to Ungar's specifications, except that his second white box was omitted. Sprague-Dawley male rats were placed in the white box and shocked for 5 s when they ran into the dark box, the passageway being closed by a barrier during the shock period. A rat which did not run back into the white box after being shocked was forced there by hand. After the first shock the rat had to be forced into the black box each time. A waiting period of 10 s separated successive trials. Five trials constituted a session, and sessions were repeated daily for 6 days. After the first few trials, most rats displayed obvious signs of apprehension (defaecation, urination, attempts to jump out, crouching in a corner) when placed in the white box. Some learned to position their paws on the grid so as to avoid shock, but the experimenter overcame this by grasping the tail (protruding under the passageway barrier) and jiggling the rat back and forth. In two experiments donors were subjected to only a single training session before decapitation 5 days later.

Recipients were Swiss-Webster mice, usually males (25–30 g). The only modification in the apparatus was the introduction of a barrier containing a mouse hole (2.7 cm diameter semicircle) in the passageway. Two measures were used: latency time to enter the hole initially; and black box time, the total spent in the black box (all four paws inside the mouse hole) in a 180 s test period.

Recipient mice received a familiarization session (day 1), then a screening session (day 2). For the 211 mice subsequently given saline or brain extracts from untrained donors, the mean values and standard errors on day 2 were: latency, 14.6 ± 0.7 s; black box time, 138.9 ± 1.4 s. The corresponding values for 172 mice later given brain extracts from trained donors were: 12.6 ± 0.6 and 139.2 ± 1.5 . We rarely found mice with black box times less than 90 s, so few were excluded. Injections (intraperitoneal) were given on day 3, testing sessions were conducted on days 4 and 5. The mice (usually twenty-four) were coded, ranked in order of day 2 latency, and assigned alternately to the treatments. All testing was "blind".

Donors were decapitated 1–2 h after their last training session. Brains were cut sagittally, sandwiched between pieces of dry ice within 30 s, and sometimes kept overnight at -20°C . Subsequently the temperature was kept below 5°C . The brains were homogenized in cold distilled water (6 ml./brain) at 850 r.p.m. using a 'Teflon' pestle. The homogenate was adjusted to pH 7.5 with NaOH and HCl, then dialysed against 20 volumes of distilled water for 24 or 48 h. In the morphine tolerance experiment the homogenate was centrifuged at $26,000g$ for 2 h and the supernatant was dialysed for 24 h. The dialysate was lyophilized, dissolved in distilled water (1 ml./brain) and stored at -20°C until use. Each recipient was given 0.5 ml. intraperitoneally. Sometimes, instead of dialysis, the homogenate was stirred on a magnetic mixer at 5°C or at room temperature, then centrifuged ($39,000g$, 30 min), and the supernatant was used directly or after lyophilizing.

The attempt to transfer tolerance to mice using tolerant rat brain extracts is summarized in Table 1. The day after receiving physiological saline or tolerant rat brain extract, all the mice reacted on the hot plate. After the test injection of morphine all displayed good analgesia, with one exception. An occasional morphine-treated mouse will react on the hot plate*, so no significance can be attached to this solitary result. There was a tendency in both groups towards enhanced morphine-induced running; the mean increase was 12% in the control group and 20% in the extract-treated group. Our

testing procedure (hot-plate analgesia and running activity) differed from that used in Ungar's laboratory^{3,10} (tail clip analgesia), and our standard test dose of morphine (35mg/kg) was much larger than theirs (4.5 mg/kg). We have shown⁹, however, that after mice are made tolerant by repeated injections, this dose fails to produce analgesia (hot plate), and opiate-induced running is substantially reduced or absent. Thus, in this experiment, no tolerance was observed in the recipient mice.

Table 1 Testing of Recipient Mice for Tolerance to Morphine

	Reaction time on hot plate (s)		Running activity (counts in 4 h)		
	Before morphine	30 min after morphine	7 days before at screening	After morphine	Change (%)
Control					
No. 15	6	NR	1,902	890	-53
24	20	NR	1,650	1,930	+14
11	14	NR	1,268	971	-23
4	15	NR	1,175	1,689	+30
19	7	NR	3,300	3,694	+11
12	10	NR	1,969	3,629	+46
21	18	NR	2,678	4,525	+41
23	7	NR	2,170	3,244	+33
					Mean + 12
Extract					
3	7	NR	1,795	1,972	+9
8	9	18	1,148	1,670	+31
6	12	NR	1,179	1,990	+41
10	9	NR	1,512	1,920	+21
17	12	NR	1,992	2,250	+12
7	10	NR	2,762	2,831	+2
13	10	NR	2,526	4,000	+37
20	7	NR	1,906	2,043	+7
					Mean + 20

Control mice received physiological saline, extract mice received brain extract from tolerant rats 24 h earlier, one half brain equivalent per mouse. Morphine sulphate (35 mg/kg free base) was given intraperitoneally. NR: no reaction in 30 s.

Two experiments were carried out with mice as both donors and recipients. Donors were made tolerant to increasing doses of the morphine congener, levorphanol tartrate (20–100 mg/kg). Controls received sodium tartrate on the same schedule. In the first experiment, many of the recipients died within a few hours of intraperitoneal administration of either type of extract equivalent to one to two mouse brains. In the second experiment, in which recipients received material equivalent to half a mouse brain, the animals survived. Three hours later, all fifteen controls and all thirteen which had received tolerant extracts reacted within 30 s on the hot plate. They were then given levorphanol tartrate (10 mg/kg of free base) intraperitoneally and were tested after 30 min. Two mice in each group reacted within 30 s; the rest showed typical opiate analgesia. Repeated testing after 1 and 2 h revealed no difference between the groups in the rate of disappearance of analgesia. In agreement with Ungar and Galvan³, therefore, we could not transfer opiate tolerance from mouse to mouse.

Preliminary to our attempts to transfer passive avoidance, we first examined the effect of foot shock on the subsequent behaviour of twenty-six untreated male mice. On day 1, half of these were individually familiarized with the apparatus for 180 s each, while the other half received shock (up to 5 s) as soon as they ran into the black box. Nearly all ran out again immediately after receiving only 1–2 s of shock. On day 2 all mice were tested again with no shock. On day 3 the unshocked animals were given saline injections and tested again on day 4. The behaviour of the unshocked mice was typical of that observed in all experiments (Table 2). On their first encounter with the apparatus there was much individual variability;

* In the screening procedure for this experiment, as noted earlier, two mice out of the thirty-four failed to display analgesia after a morphine injection.

mean latency was 61 s (s.d. 52; range 8–180, with one mouse not entering the black box at all). On day 2 the mean latency had decreased to 16 s and the responses were more consistent (s.d. 16, range 4–64). The same trend continued on day 4. Black box times showed similar effects, with means increasing from 88 to 146 to 157 and s.d. decreasing from 41 to 23 and 24. Thus, after the familiarization day, both latency and black-box times were predictable and reliable, with no remarkable effect from the inert injection on day 3. In contrast, the shocked mice showed a mean latency increase from 33 s to 139 s, and nine of them avoided the black box altogether. The differences between the unshocked and shocked groups were highly significant (t test, two-sample rank test, binomial sign test).

In the transfer experiments there were no evident differences among the several procedures used, so data are pooled for presentation. To minimize variability, each mouse was used as its own control. The results are expressed as a frequency distribution of differences in latencies (Fig. 1) or black-box times (Fig. 2) between day 2 and day 4 and between day 2 and day 5. The null hypothesis predicts a mean difference of zero, but in the controls there is a consistent decrease in latency and increase in black box time, which presumably reflects greater familiarity with the box. For the 211 control mice and the 172 mice receiving extracts from trained donors the frequency distributions are virtually identical.

In spite of negative results for most of the animals, in some

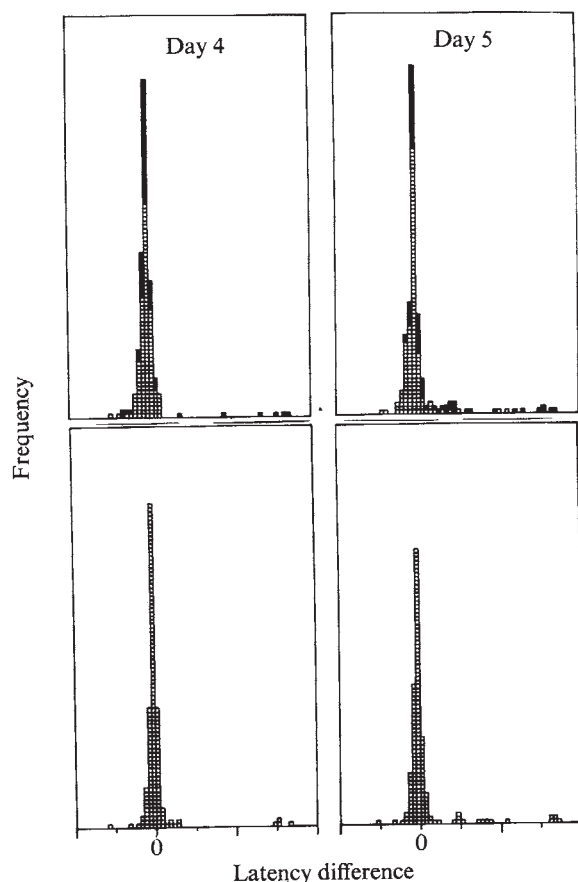


Fig. 1 Frequency distribution of latency differences in eighteen experiments. Ninety-three trained donor rats; seventy-three untrained donor rats; 146 saline injected recipient mice; sixty-five recipient mice receiving brain extract from untrained donor rats; 172 recipient mice receiving extract from trained donor rats. Each box represents one mouse. In upper graphs open boxes are saline controls, black boxes are untrained brain extract controls. In lower graphs all mice received extract from trained donors. Horizontal axis shows change in latency for each mouse from day 2 (screening) to day 4 (or day 5). Each large scale mark represents 100 s; thus the range shown is from -100 to +200 s.

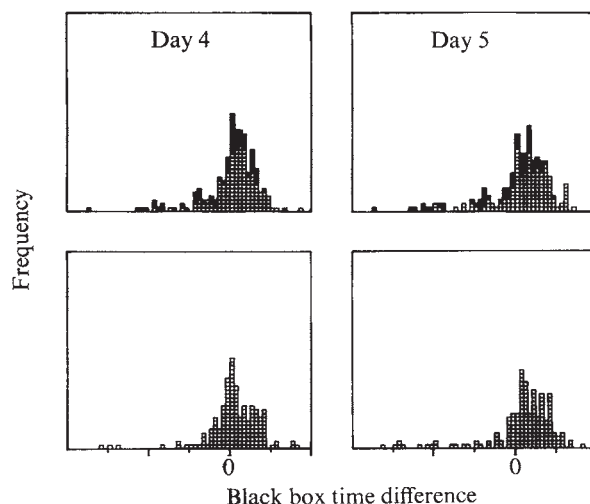


Fig. 2 Frequency distribution of black box time differences in eighteen experiments. Symbols and scales as in Fig. 1.

mice one can find possible indications of a weak effect in the relevant direction. Latency results on day 4 show only two of the controls having an increase greater than 100 s, whereas seven of the extract recipients fall into this category. On day 5 the corresponding figures are eight and ten. For black box time on day 4, only two control mice but six extract-treated animals show reductions of more than 100 s. On day 5 the corresponding figures were six and twelve.

Table 2 Effects of Shock and No Shock on Latency and Black Box Time

	Latency (s)			Black box time (s)		
	Median	Mean	s.d.	Median	Mean	s.d.
Unshocked group (N=13)						
Day 1	53	61	52	98	88	41
Day 2	11	16	16	150	146	23
Day 3		Injection			Injection	
Day 4	8	12	11	166	157	24
Shocked group (N=13)						
Day 1 (shocked)	30	33	23	—*	—*	—*
Day 2	180	139	70	0	34	61

* No data because mice ran out immediately on being shocked.

Mice were treated as described in the text. Foot shock (80 V a.c.) was administered on day 1 as soon as a mouse entered the black box. Unshocked mice were given a saline injection intraperitoneally on day 3 and retested on day 4.

To test the hypothesis that a few mice were sensitive to the effect of the extract, we examined the extremes of the frequency distributions. In each experiment we noted which three mice had the longest latencies, and which three had the shortest black box times. The results were: latency day 4, twenty-six extract and twenty-four control; latency day 5, twenty-eight and twenty-three; black box time day 4, twenty-one and twenty-eight; black box time day 5, twenty-seven and twenty-two†. There is a very small non-significant trend in the predicted direction.

Dr Ungar kindly supplied us with some of his extract from trained and untrained donors to test in our laboratory. For this experiment fifty-two mice were familiarized, screened, ranked and assigned to treatments as described earlier. One

† The totals sometimes exceed $3 \times 16 = 48$ because if the third-ranking mouse was one of a tied pair, both members of the pair were included.

of us injected the animals, another did all the testing "blind". At the screening session, the mean latencies were: group A = 12 s, group B = 12 s; mean black box times: group A = 145 s, group B = 142 s. Injection dose was 1 g rat brain equivalent/mouse, on Dr Ungar's recommendation (Table 3). In both groups the medians and means indicate a skewing of the frequency distributions towards longer latencies and shorter black box times. The only suggestion of a difference between the groups was at 24 h, where there was a slight (but not significant) preponderance of longer latencies and shorter black box times in group B.

Table 3 Effect of Extract from Untrained and Trained Donors

Test time after injection (h)		Latency change (s)			Black box time change (s)		
		Median	Mean	s.d.	Median	Mean	s.d.
6	A	-4	-1	7	16	5	42
	B	0	5	19	4	1	27
24	A	0	13	39	3	-1	41
	B	2	30	55	10	-10	57
48	A	4	27	59	0	-13	62
	B	3	34	67	12	-11	70
		Extreme latency change (> 50 s)			Extreme change in black box time (> 50s)		
6	A	0			2		
	B	2			1		
24	A	1			1		
	B	5			4		
48	A	3			3		
	B	4			4		

Extracts were supplied by Dr Ungar and tested as described in text. Data in the upper part of the table are changes in latency and black box time (result at time of testing less than at screening session), computed for each individual mouse. Medians, means, and standard deviations are for the set of individual mouse differences. Group A received extracts from untrained donors, group B from trained donors. There were twenty in each group. Data in lower part of table are numbers of mice displaying extreme behaviour in the predicted direction (increased latency, decreased black box time).

When we did not obtain positive results with Ungar's extracts in our laboratory, we concluded that our mouse strain rather than our technique of preparing extracts might be at fault. With Dr Ungar's cooperation, we shipped 100 males of our strain (Bioscience Labs, Inc.) to Houston, where they were tested at various doses of extract along with his Texas inbred strain. Because we were unable to agree with Dr Ungar on the interpretation of the results they are not included here but will presumably be published independently by him.

Finally, although most mice were not good recipients, what few weak effects had been observed might be attributable to a small fraction of the mice having recipient capacity. A transfer factor might be destroyed enzymatically in most recipients, but not in genetic variants. Accordingly, we saved those males and females which showed the greatest prolongations of latency or reductions in black box time in all sixteen experiments. These were bred, and eventually the F1 generation was tested, by randomly assigning littermates to the comparison groups. At screening, results were: mean latencies 12 (s.d. 5) and 11 (s.d. 5), black box times 134 (s.d. 24) and 132 (s.d. 27), for the groups that were to receive extract from untrained and trained donors, respectively. There were no significant differences between the two extracts (Table 4).

We should not dismiss the possibility that acquired behaviour, such as conditioned dark avoidance, can be transferred by brain extracts merely because the proposed mechanisms^{11,12} seem fanciful, especially since confirmatory reports have been published from several laboratories¹³⁻¹⁵. On the other hand, our rather exhaustive but unsuccessful attempts to replicate the findings suggest, at the least, that the precise conditions

Table 4 Testing of F₁ Offspring of Best Recipients

Test time after injection (h)		Latency change (s)			Black box time change (s)		
		Median	Mean	s.d.	Median	Mean	s.d.
24	Untrained extract	0	0	7	-2	-10	43
	Trained extract	-2	-1	6	16	11	27
48	Untrained extract	-2	-1	8	21	18	18
	Trained extract	-2	-2	6	10	5	37

Offspring of best recipients from sixteen experiments. Five litters were screened, then littermates assigned randomly to the two groups. N = 14 for each group. Extracts from untrained and trained donors were prepared as described, and injected.

for successful transfer will have to be worked out and defined much more carefully than heretofore if this phenomenon is to meet the test of reproducibility. As matters stand now, it would be more useful for the proponents to establish rigorously and in great detail the conditions for reliably transferring one kind of behaviour in a single species, than to publish marginally successful experiments using different paradigms in a variety of animals.

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Formation of Cerebrospinal Fluid in Spinal Subarachnoid Space

It has been generally accepted that the fluid bathing the central nervous system is produced within the cerebral ventricular system, although some people have favoured extra-ventricular formation¹⁻³ as well. Some considered that another site of formation was the spinal subarachnoid space^{4,5} (Fig. 1), but others disagreed^{6,7}.

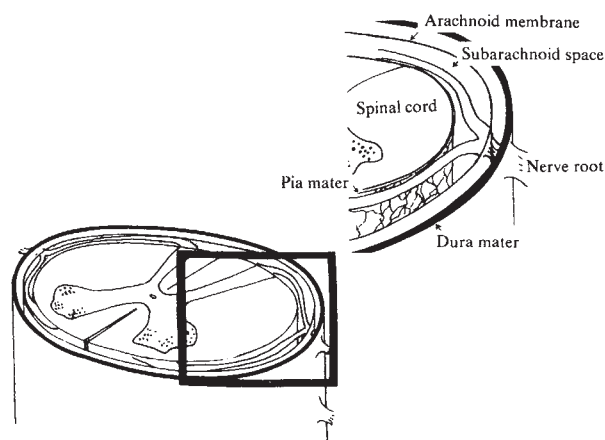


Fig. 1 Section through spinal cord showing position of subarachnoid space.

We have now investigated this problem, performing the inulin perfusion through the spinal subarachnoid space of mongrel dogs (12–15 kg). For anaesthesia 30 mg of pentobarbital/kg body weight was given intraperitoneally and each dog was endotracheally intubated and connected to a respirator after receiving 20 mg of succinylcholine intramuscularly to keep it still.

The dog was fixed in a prone position with the head slightly elevated. Laminectomies were performed over the cauda equina and upper cervical region. The lumbosacral subarachnoid space was punctured with an Argyle 'Medicut' cannula, which was connected to an inflow tubing through which passed an artificial CSF containing 25 mg% of inulin at a rate of about 0.3 ml./min. An Argyle cannula was also inserted into the upper cervical subarachnoid space and connected with outflow tubing which could be raised or lowered to change the CSF pressure during the experiment. No CSF was allowed to leak in and around the puncture sites.

Outflow samples were obtained every 10 min, after a steady state had been reached, at different pressures. These were analysed by the resorcinol recovery method for inulin concentration determination.

Every experiment was followed by autopsy to see whether the perfusion fluid was limited in the spinal subarachnoid space and whether it was bathing the entire system. This was confirmed by perfusing methylene blue dye in the same condition immediately after the final sample had been collected. We found no mixing of subarachnoid fluid between intracranial and spinal subarachnoid spaces.

CSF formation rate was calculated by the method of Heisey *et al.*¹, combining the effects of hydrostatic pressure on outflow–inflow differences and on clearance of inulin, which measured only bulk absorption. The notation to be used in the following description is that of Heisey *et al.* The use of clearance of inulin as a measure of absorption was based on the assumption that the diffusive absorption of inulin was negligible. Therefore the relation between inulin clearance and bulk absorption can be written

$$\dot{V}_a = C_{in} \quad (1)$$

where $\dot{V}_a$ is the rate of bulk absorption in ml./min; C_{in} is the steady state clearance in ml./min of inulin out of the perfusion system per unit concentration; $C_{in} = \dot{V}_i C_i - \dot{V}_o C_o / \bar{C}$; $\bar{C}$ is mean concentration in the system $= C_o + 0.37 (C_i - C_o)$; $\dot{V}_i$ and $\dot{V}_o$ are the influx and efflux flow rates in ml./min, and C_i and C_o are the concentrations of inulin in the influx and efflux respectively.

When the outflow volume was equal to the inflow, the rate

of absorption was equal to the CSF production rate ($\dot{V}_f$). Therefore, the relation between outflow–inflow difference, formation and absorption of CSF can be expressed as

$$\dot{V}_o - \dot{V}_i = \dot{V}_f - \dot{V}_a \quad (2)$$

Substituting $\dot{V}_o - \dot{V}_i$ for O–I and $\dot{V}_a$ for C_{in} , from equation (1), the formation rate can be calculated by the following equation.

$$\dot{V}_f = (O - I) + \dot{V}_a \quad (3)$$

The outflow rates are therefore less than inflow rates, and more fluid is absorbed than is produced. The outflow rate increased as the outlet was lowered and decreased when it was raised. In spinal subarachnoid perfusion, expressions for

O–I, $\dot{V}_a$ and $\dot{V}_f$ are:

$$O - I = 0.0206 (\pm 0.00165) - 0.348 \times 10^{-3} \text{ CSFP ml./min}$$

$$\dot{V}_a = 0.0005 (\pm 0.00005) + 0.320 \times 10^{-3} \text{ CSFP ml./min}$$

and

$$\dot{V}_f = 0.0201 (\pm 0.00244) - 0.288 \times 10^{-4} \text{ CSFP ml./min} \quad (n = 41)$$

where CSFP stands for cerebrospinal fluid pressure when zero pressure was taken at the level of the spinal cord during the perfusion. From these equations the production rate is 0.018 (± 0.002) ml./min when outflow is equal to inflow rates at the pressure of 59 mm of water.

Although the results give no data as to the actual site or mechanism of the formation of fluid in the space, these experiments have conclusively demonstrated that CSF is being formed in the spinal subarachnoid space and that it is important for this extraventricular production of fluid to be recognized, particularly when one considers the problem of the CSF circulation.

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Reflecting Spheres in the Eyes of Weakfishes (Sciaenidae)

A REFLECTING layer or tapetum lucidum occurs in the eyes of many fishes; in teleosts, it lies in the pigment epithelium¹. The reflecting material is usually considered to be guanine, but in weakfishes or seatrouts (Sciaenidae) it is a lipid^{2,3}. We have been able to characterize the lipid material.

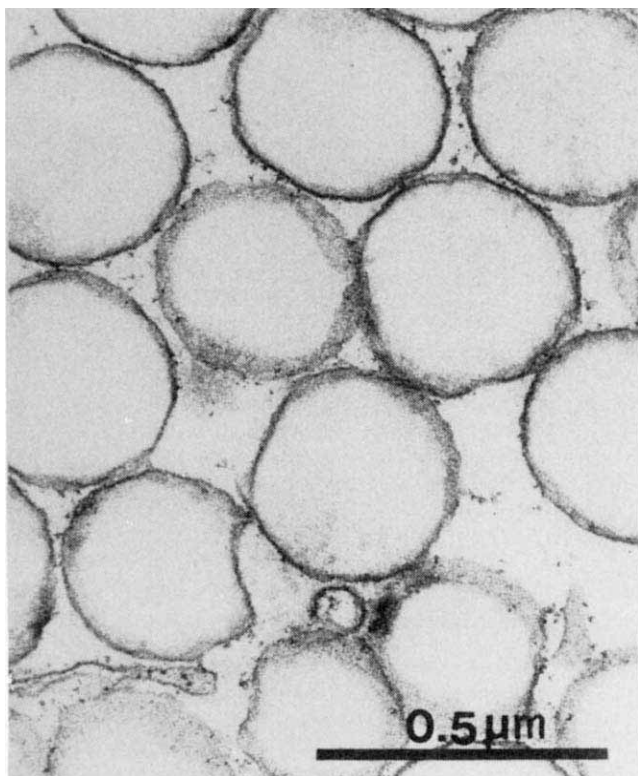


Fig. 1 Lipid spheres in a pigment epithelial cell of the sandtrout (*C. arenarius*) fixed in 3% glutaraldehyde in a 0.1% phosphate buffer, postfixed in 2% osmium tetroxide in the same buffer. This fixation technique does not preserve all the contained lipid, but does define the size and shape of the spheres. The boundary layer is associated with some of the contained lipid and appears to be thicker than an ordinary cytomembrane. Other fixation techniques retain the lipids².

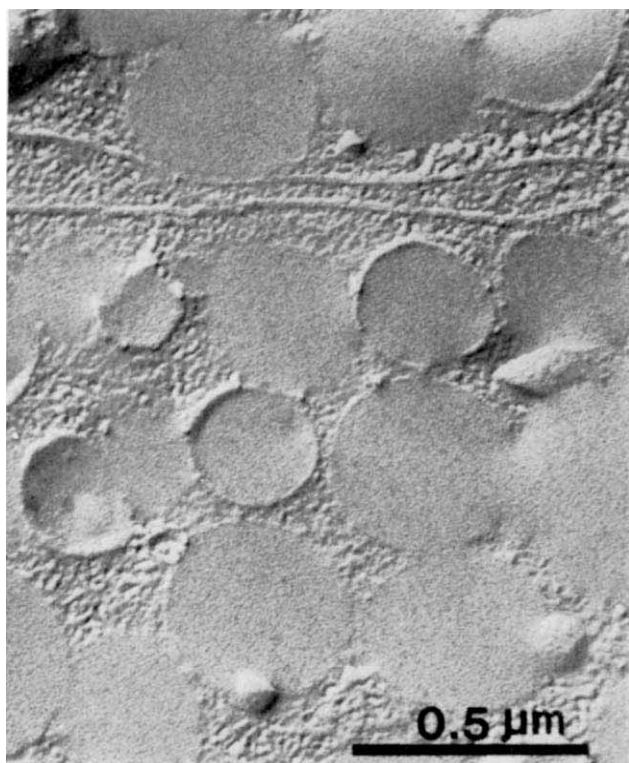


Fig. 2 Lipid spheres from the pigment epithelial cells of the sandtrout (*C. arenarius*) as they appear in freeze-etch preparations. Note the plasma membranes of two pigment epithelial cells and compare these cytomembranes with the surface of the lipid spheres.

The pigment epithelial cells of seatrouts (*Cynoscion arenarius* and *C. nebulosus*) are densely packed with small spheres, about 0.4 μm in diameter (Fig. 1), which are present throughout the length of the cell, but most abundant distal (that is, vitread) to the nucleus. There are about fifteen spheres per μm^3 . The cytoplasm in the terminal process of the pigment epithelial cells contains no other visible structures, apart from small vesicles and occasional membranes, when the eye is dark-adapted and the retinal pigment withdrawn. Each sphere is surrounded by a thin membrane, visible as a fine dark line in electron micrographs when part or all of the lipid content of the sphere has been dissolved away. When the material was examined by the freeze-etch technique, the membrane could often be seen as a separate structure, cleaving differently from the internal component. The freeze-etch electron micrographs (Fig. 2) also provided confirmatory evidence of the shape, dimensions, and distribution of the tapetal spheres. The membrane is not a "unit membrane", but is similar to those associated with some plant lipid bodies⁴⁻⁶, and in lymph chylomicrons⁷. The membranes surrounding the lipid spheres (bodies) in the corn root were termed "boundary membranes" by Trelease⁶ who was able to observe them both *in situ* and after isolation. The tapetum has a fairly high refractive index, judged to be close to n_D^{23} 1.50, as determined by the immersion method using Thoulet's solution.

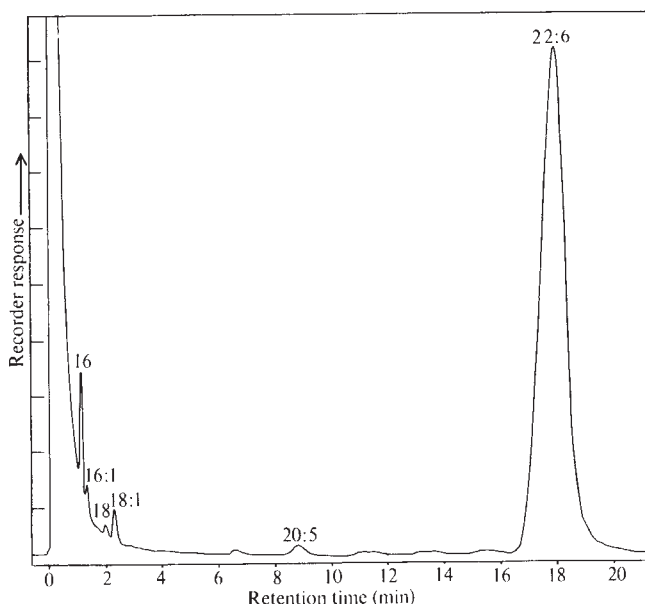


Fig. 3 Gas-liquid chromatograph of a transesterified sample of lipid from the tapetum lucidum of the sandtrout. Operating conditions were: Perkin-Elmer gas chromatograph, model 880; the analysis was carried out on a column of copper tubing, 6 feet $\times$ 1/8 inch, packed with 'DEGS' 10% on 'Gas-Chrom Z' (mesh size 60/80); carrier gas was helium, flow rate was 45 ml./min; the column was operated isothermally at 180° C.

To characterize the reflecting material, the pigment epithelium was removed from the eyes of dark-adapted fish; the material contained the entire pigment epithelial cell, including the white reflecting material, melanin, and other cellular organelles. It was cytolysed in distilled water and lightly centrifuged, and the supernatant contained the white reflecting material which was then extracted in chloroform and examined by thin-layer chromatography. It consisted almost exclusively of one major component running with triglycerides. Extracts of the entire pigment epithelium and whole retina contained the same principal component running with the triglycerides. Isolated retina did not contain a large triglyceride component. To secure larger amounts of material, therefore, whole retinas

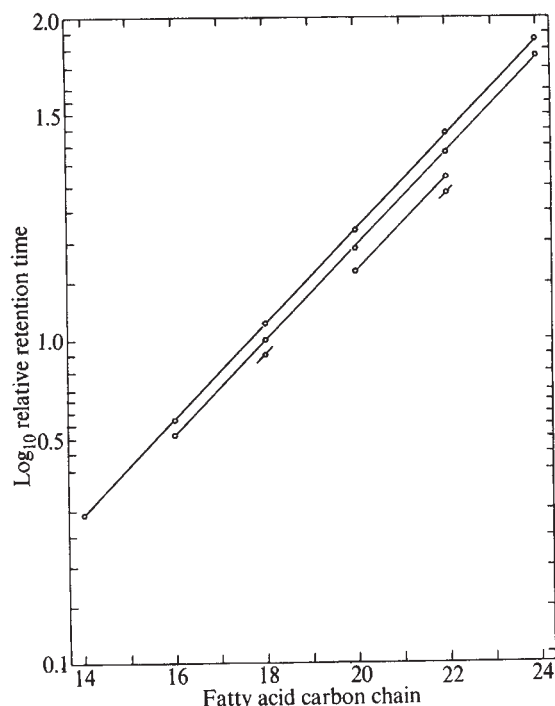


Fig. 4 Plot of $\log_{10}$ retention times against carbon number of fatty acid methyl esters and of the main component from the tapetum lucidum of the sandtrout. Retention times relative to methyl octadecaenoate. Lines from above downwards, saturates, monoenoic, dioenoic (n-C18:2) only, pentaenoic, hexaenoic (authentic n-C22:6 and sample of tapetum). Data from a gas-liquid chromatograph record of a standard mix co-injected with a sample of unknown. Operating conditions: column of copper tubing, 5 feet $\times$ 1/8 inch packed with 10% 'Apiezon-L' on 'Gas-Chrom Z', carrier gas helium, flow rate 45 ml./min, isothermal run at 225°C.

Confirmation was offered by a plot of log retention times against carbon number (Fig. 4). Further evidence for the chain length was provided by hydrogenation after which more than 95% of the fatty acid was converted to C22:0.

The evidence indicated that the reflecting material was a triglyceride containing docosahexaenoic acid. The original purified extract ran at the same rate on thin-layer chromatography as tridocosahexaenoin, and infrared spectra of sample and of authentic tridocosahexaenoin were closely similar (Fig. 5). It was concluded that the reflecting material consisted largely or almost exclusively of tridocosahexaenoin.

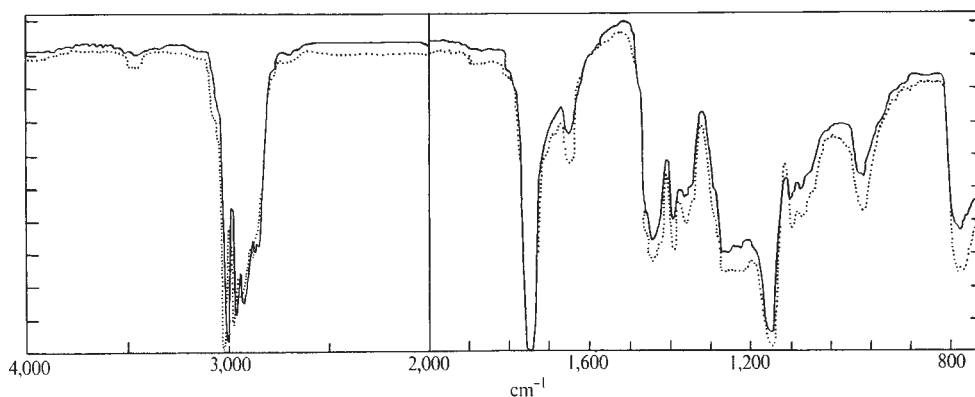
The occurrence of a simple unmixed triglyceride in a tissue is unusual; in depot fats of invertebrates and fishes, triglycerides are usually asymmetric, and n-C22:6 occurs preferentially in position 2 (proportionality constant $y_2 = 2.06\chi$, where $\chi = \text{mol\% } 22:6$ in the total triglycerides¹⁰).

The refractive index measurement of tridocosahexaenoin is not available, but that of fatty acid n-C22:6 is 1.49, and that of tridocosahexaenoin may be close to it.

The extract is transparent and very refractile; the diameter of the lipid spheres is about one wavelength (of visible light) and they act as Mie scatterers. Measurements and calculations (to be presented elsewhere) show that the tapetum is an efficient diffuse reflector. The system allows the fish to use light more efficiently in dim illumination, when rod vision or twilight vision is operative. Seatrouts along the coast of the Gulf of Mexico live in shallow waters containing a great deal of suspended material, and often encounter low levels of illumination during daylight hours. Light which passes through the photoreceptor layer of the retina without being absorbed is reflected back into the photoreceptor zone by the lipid spheres in the pigment epithelial cells. This second passage through the photoreceptors allows more light to be absorbed and seems to be effective in promoting vision in dim light conditions.

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Fig. 5 IR spectra of lipid from the tapetum lucidum of the sandtrout (continuous line) and of authentic tridocosahexaenoin (Hormel Institute, estimated purity >90%, interrupted line). Spectra obtained on a Perkin-Elmer grating infrared spectrophotometer, model No. 237B. Solvent, carbon tetrachloride in sodium chloride cells. Fast scan.



including the pigment epithelium were collected (fifty eyes). They were extracted in chloroform-methanol by the method of Folch *et al.*⁸, and the major component was isolated by column and preparative TL chromatography⁹. The eluant, examined by thin-layer chromatography in three different solvent systems, resembled triglycerides in chromatographic behaviour, and was well demarcated from phospholipids, sphingomyelin, cerebroside, mono- and dipalmitins, cholesterol, cholesteryl esters, waxes, and diacyl glyceryl ethers. It was hydrolysed, esterified or transesterified and the presumptive methyl esters examined by gas-liquid chromatography on four different columns, ranging from non-polar to very polar (FFAP on 'Gas-Chrom Q', 'Apiezon-L' on 'Gas-Chrom Z', 'DEGS' on 'Gas-Chrom Z', and 'EGSS-X' on 'Gas-Chrom P'). The sample showed some minor peaks corresponding to various methyl esters of fatty acids from C14 to C22:5, and one major peak corresponding to n-C22:6 (Fig. 3). The latter comprised 95% or more of the fatty acids present.

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litter weight results is that fungi of the mycorrhizal partnership, having a biological advantage because they are not dependent on litter for an energy source, become dominant in the litter and suppress the activity of saprophytic organisms. Litter decomposition slows down and conditions favouring mor formation result.

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Mycorrhiza and Litter Decomposition

FORMATION of the mor type of forest litter, characterized by slow decomposition, has been attributed to a number of factors¹⁻³, but the effect of mycorrhizal fungi, though postulated^{4,5}, has not been studied experimentally. We have conducted an experiment in which root activity and thus mycorrhizal activity were reduced in small areas of an unthinned *Pinus radiata* stand. The following treatments (three replicates in a randomized block design) were applied to 1 × 1 m plots sited between the trees.

(A) The plot boundary was cut to a depth of 30 cm at commencement and recut every 2 weeks. All litter was removed to a sheet of hessian with minimum disturbance. As many roots as possible were removed from the soil to a depth of 30 cm and the litter was carefully replaced.

(B) The plot boundary was cut as above and the litter was removed. The soil was dug over to a depth of 30 cm but no roots were removed. The litter was replaced.

(C) The plot boundary was cut as above. The plot was not dug, nor were any roots removed. The litter was removed and replaced.

(D) The control plot was neither dug nor cut, but the litter was removed and replaced.

Table 1 Dry Weight of Litter from Sample Plots after 12 Months

Treatments			
A	B	C	D
474.8 ± 211.5	351.6 ± 133.7	659.0 ± 328.4	1,681.7 ± 213.9

The figures represent the means (in grams) of three replicates ± the standard deviation.

After 12 months the weight of litter per plot which had received treatment D was significantly ($P < 0.001$) greater than in those given treatments A, B and C. Experimental design ruled out the effect of soil disturbance or decomposition of severed roots. It seems clear that the presence of living roots suppressed litter decomposition in some way. *Pinus* is a highly mycotrophic genus⁶, and for practical purposes *P. radiata* roots in the field may be considered as mycorrhizas. The observed effect could be a result of some physiological activity of the tree roots functioning as a separate entity. On the other hand, it was observed that basidiomycetous mycelial wefts, normally very conspicuous in the litter, were completely absent from plots which had received treatments A, B and C although they were obvious in plots given treatment D. We suggest that these wefts were the external mycelium of the mycorrhizas. An alternative explanation for the

Immunological Suppression of the Occurrence of Spontaneous Mammary Tumours in C3H/He Mice

MICE immunized with syngeneic tumours acquire resistance to subsequent transplantation of mammary tumours¹⁻³, but there have been no reports of the immunological suppression of the spontaneous occurrence of the tumours by the use of appropriate specific antigens. Mouse mammary tumours appear in favourable conditions in a high percentage of both virgin and bleeding female C3H mice⁴. Mice of the C3H/He strain are genetically pure (syngeneic) and acquire the mammary tumour inducing virus (MTV) by vertical transmission.

We have assumed that the occurrence of spontaneous mammary tumours in C3H/He mice would be suppressed by immunization with the specific antigens of the mammary tumour.

The tumour specific transplantation antigen (TSTA) of mouse mammary tumours can be extracted from MM102 cells—the ascitic line derived from a spontaneous mammary tumour in a C3H/He mouse⁵. The TSTA extracted from MM-

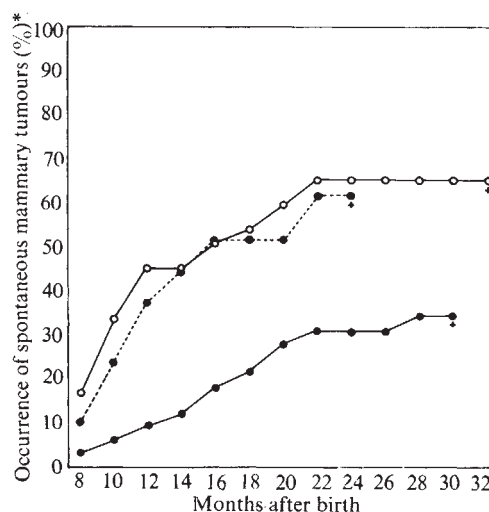


Fig. 1 Occurrence of spontaneous mammary tumours in C3H/He mice. Each plot is figured out the total occurrence within the months. —●—, Immunized with MM-antigen; ---●---, treated with C3Hf organ extract; —○—, untreated. +, All mice were dead by the marked month.

102 cells is termed MM-antigen. C3H/He mice immunized with MM-antigen acquire resistance to the transplantation of MM102 cells⁵ and antibodies which specifically elicit immunological cytotoxicity on MM102 cells can be demonstrated in both the sera and the lymphatic cells of the resistant mice⁶. Further, two experiments suggest the existence of an antigen common to spontaneous mammary tumour cells and MM-antigen. Electron dense precipitates on the cell surface of the microvilli of spontaneous mammary tumour cells treated with RMS (syngeneic antiserum against MM-antigen) can be seen under the electron microscope, using the peroxidase labelled antibody method⁷; and immune cytotoxic activity of RMS can be demonstrated in primary tissue culture cells of mammary tumours⁸. It therefore seemed possible that spontaneous mammary tumours in C3H/He mice might be suppressed by immunization of these mice with MM-antigen.

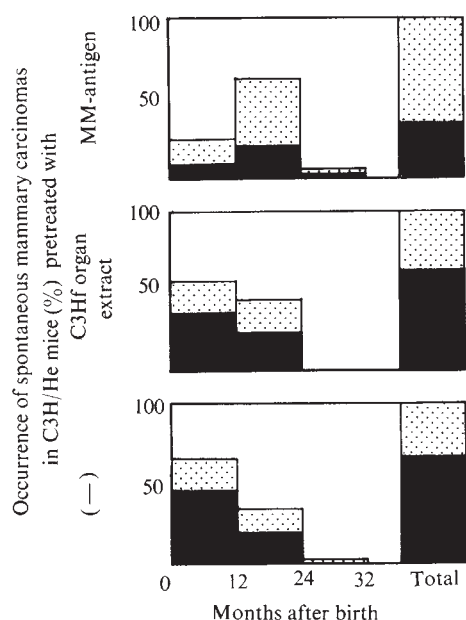


Fig. 2 Occurrence of spontaneous mammary tumours in C3H/He mice. The results are summarized at the end of each year. Shaded bar indicates the percentage of mice with spontaneous mammary tumours. Stippled bar indicates the percentage of dead mice not bearing the tumour.

We divided 105 C3H/He mice about 8 weeks old into three groups of thirty-five. Mice of the first group were immunized with MM-antigen. The visceral organs apart from the lymphatic organs of C3Hf mice were treated beforehand with the same method as extracting MM-antigen from MM102 cells (C3Hf mice are syngeneic with C3H/He mice but do not possess MTV). The second group of mice was immunized with this extract. The third group of mice was untreated. Immunization was carried out as follows. On the first, third and fifth days the mice of the first group were injected intraperitoneally with 0.5 ml. of 1 : 100 dilution of the MM-antigen (1 ml. of the undiluted antigen used here was extracted from 2×10^7 MM102 cells). On the seventh, ninth and eleventh days the mice were injected with 0.5 ml. of 1 : 50 dilution of the antigen. On the thirteenth, fifteenth and seventeenth days the mice were injected with 0.5 ml. of 1 : 25 dilution of the antigen, and on the nineteenth day the mice were injected with 0.5 ml. of 1 : 10 dilution of the same antigen. The mice were then boosted with 0.5 ml. of the undiluted MM-antigen once a month for 3 months. Immunization of the mice ended within 6 months after birth. The injection of extract from C3Hf organs into mice of the second group was carried out by the

same method as that of MM-antigen into the mice of the first group.

In the sera of the mice in the first group the antibody reacted specifically with MM102 cells; this could be demonstrated by the immune adherence method until the thirteenth month after the end of immunization, although the antibody titre gradually decreased.

All mice were given dry feed and water *ad libitum* in the same conditions. Three mice from the first group and six mice from the second died during the immunization process, and were omitted from all calculations. The mice were examined for spontaneous mammary tumours once a week after the end of the immunization schedule.

The occurrence of the tumours in the first group was considerably suppressed compared with the second and the third groups (Fig. 1). No significant differences appeared between the second and the third groups. All the results (Fig. 2) were summarized at the end of each year for a period of 3 years. The suppression within the first year was quite drastic in the first group. There was no significant difference in deaths from other causes in the first year. The total occurrence of the tumours over 3 years in the first group was approximately one half of those in the second and third groups.

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Chromosome Polymorphism in American Negro and White Populations

THE biological and clinical implications of variations of human chromosome lengths are poorly understood.

In the study we now report, minor variations in chromosome length were correlated with clinical and sociological data obtained from each patient for 4,482 consecutively born infants. By far the strongest correlations were between certain minor chromosome variants and race. Most variants showing a racial difference of frequency were twice as common in Negroes as in Caucasians.

The human variants can be considered as polymorphisms as in *Drosophila* and other species¹⁻³ because (1) they occur at relatively high frequencies; (2) they are usually associated

with normal phenotypes and (3) they are frequently inherited from generation to generation^{4,5}. Racial variation of the length of the *Y* chromosome⁶, and the question of racial variation in the size and morphology of the short arms of the chromosomes in groups *D* and *G*⁷ have already been discussed.

All infants of more than 20 weeks gestation born at Yale-New Haven Hospital from October 1967 to October 1968 were included in the study and good preparations were obtained from the cord blood of 4,366 of 4,482 newborns^{8,9}. Cells were treated with 0.075 M KCl and stained with orcein. Two karyotypes were examined from each infant and variation in length was scored using callipers. Variants were detected by comparing the lengths of individual chromosomes against internal standards within each cell (Table 1) and were recorded only when present in both cells. A long *Y* chromosome, for example, was recorded if the *Y* chromosome in both cells was greater than or equal to *F*19 in length, and an unusually long *Y* recorded if it was greater than the total length of *E*18 (Fig. 1). All the results are based on samples of two cells per individual. Studies with larger samples show that, in general, two-cell ascertainment are correct except for the commoner variants. The frequencies of $Y \geq 19$, short arm of *D* or *G* = *E*18 short arm, and both *E*16 variants were, however, overestimated, and the frequencies reported in Table 1 should not be used to estimate the actual frequencies of these variants.

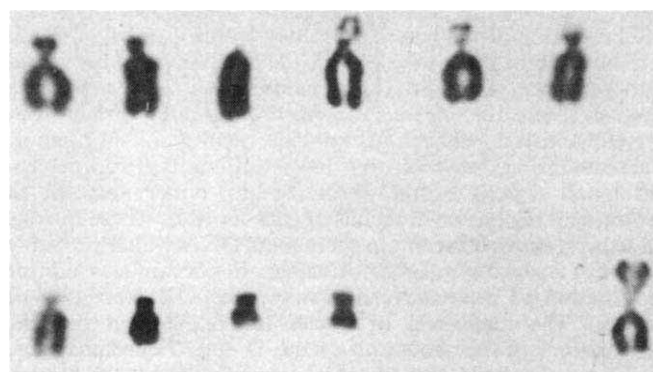


Fig. 1 Minor chromosome variants. In the top row, six polymorphisms in group *D* are shown (left to right): very long short arm $\geq$ *E*18 short arm, long short arm = short arm *E*18, deleted short arm, long streaked satellites, large satellites ($>$ short arm), and tandem satellites. *Y* chromosome polymorphisms in the bottom row are: $Y > E18$, $Y > F19$, $Y < G22$, *Y* metacentric. An example of a metacentric *C* chromosome with a secondary constriction adjacent to the centromere is shown at the far right, bottom row. Conventional staining with orcein.

Table 1 Frequencies of Polymorphisms in Caucasian and Negro Infants

Type of variation	Children of 3,476 Caucasian mothers		Children of 807 Negro mothers		<i>P</i>
	<i>N</i>	%	<i>N</i>	%	
Increased secondary constriction region <i>A</i> 1	8	0.23	3	0.37	ns
Unusually metacentric <i>C</i> chromosome	2	0.06	10	1.24	<0.01
Short arm of a group <i>D</i> chromosome:					
Increased length (= <i>E</i> 18 short arm)	480	13.80	171	21.18	<0.01
Increased length ($>$ <i>E</i> 18 short arm)	0	0.25	7	0.87	<0.01
Large satellites ($>$ short arm same chromosome)	78	2.24	34	4.21	<0.01
Tandem satellite	3	0.09	2	0.25	nt
Streaked satellite	1	0.03	1	0.12	nt
Short arm absent	2	0.06	0	0	nt
Variations in long arm of <i>E</i> 16:					
Increased length ($>$ short arm <i>C</i> 6)	71	2.04	32	3.96	<0.01
Decreased length (<i>E</i> 16— <i>E</i> 18)	19	0.55	3	0.37	ns
Short arm of a group <i>G</i> chromosome:					
Increased length (= <i>E</i> 18 short arm)	81	2.33	45	5.58	<0.01
Increased length ($>$ <i>E</i> 18 short arm)	2	0.06	0	0	nt
Large satellites ($>$ short arm same chromosome)	92	2.65	36	4.46	<0.01
Tandem satellite	3	0.09	0	0	nt
Streaked satellite	1	0.03	0	0	nt
Variations in length of the <i>Y</i> chromosome in 1,741 Caucasian and 411 Negro males:					
Y long ($>$ <i>F</i> 19)	253	14.53	60	14.59	ns
Y long ($>$ <i>E</i> 18)	6	0.34	1	0.24	ns
Y short ($<$ <i>G</i> 22)	7	0.40	2	0.48	ns
Y metacentric	2	0.11	0	0	nt
No polymorphism	2,131	61.31	389	48.20	<0.01

nt, Not tested because of small *N*. ns, χ^2 test for difference in frequency between Caucasian and Negro infants not significant (>0.05).

The most marked difference between races occurred in the frequency of metacentric *C* chromosomes (Fig. 1). This chromosome was observed in only two of 3,176 Caucasian children but was a relatively common variant in Negro children,

occurring in ten of 807 children (1.2%, a twenty-fold difference). In every case the chromosome was characterized not only by an unusual degree of metacentricity but a secondary constriction adjacent to the centromere (Fig. 1). All the children were phenotypically normal except for one of the two Caucasian children who had a birth weight below 2,500 g. Precise identification of this chromosome is difficult with conventional staining, but in two cases restaining with quinacrine mustard⁹ showed it to be chromosome *C*9. Of sixteen newborns with a short arm of a *D* chromosome greater than the short arm of *E*18, seven were Negro and nine Caucasian, a four-fold difference in frequency. The most common autosomal minor variants occurred twice as often in Negro children as in Caucasian children (Table 1). The common denominator in each of these seven variants was the presence of a secondary constriction in each of the involved chromosomes. Moreover, each of the acrocentric short arm variants is believed to share a common function of nucleolar organization and association, unlike the metacentric *C* and *E*16 variants, although secondary constrictions are present in both.

The relative differences in frequencies in the *D* and *G* group between Caucasian and Negroes were consistent with those found in the smaller sample of twenty Caucasian and twenty Negro children reported by Starkman and Shaw⁷. Similarly, the earlier work by Cohen and Shaw⁶ did not show a difference between twenty Caucasian and twenty Negro adults, although a long *Y* chromosome occurred more commonly in twenty Japanese males. Data are presented in this study only in Caucasian and Negro infants because the number of other infants included in the study was small, but of nine unusually long *Y* chromosomes ($Y > 18$) one occurred in a Chinese baby and one in a child of Turkish parents. Only one Turkish child and nine male Chinese infants were included in the study. The clinical significance of these minor variants is also under study and in at least one case a statistically significant correlation has been established: the presence of a *Gp*(sat)+ variant in Caucasian infants was associated with a two-fold increase in the frequency of low birth weight⁸. Certain of the rare variants may also carry an increased risk in Caucasian infants⁹. Racial polymorphisms, however, seem to be the most common correlate of chromosomal variation.

The relative frequencies of the variants may represent an equilibrium between their rates of generation in and elimination from the two populations. The lower frequency of chromosome polymorphism in the Caucasian population may be related to the apparently higher risk of phenotypic abnormality associated

with certain of the polymorphisms in Caucasians. The rate of elimination of the polymorphism from the Caucasian population would be more rapid, hence the equilibrium value would also be lower. Such an argument implies a similarity in the genetic effects for the numerous chromosomal variants observed, and it is difficult to imagine such a unifying factor. It is interesting, however, that most variants occur in heterochromatic regions of the chromosomes, either adjacent to centromere regions or in the distal part of the Y. These regions have been shown to contain redundant DNA which probably does not code for mRNA. Changes in the amount and/or distribution of heterochromatin may affect the expression of genes. The regulation of nucleolar organization regions, which occur in the short arm of the D and G chromosomes, may be particularly affected. It is also possible that the heterochromatin is basically different in the two populations or that differences in the genetic backgrounds respond to similar modifications of heterochromatin in different ways. More definite statements may be possible after examination of the variant chromosomes using newly developed techniques for the visualization of heterochromatin¹¹⁻¹⁴.



Fig. 2 Increased centromeric heterochromatin in chromosome A1. A1 homologous chromosomes from an individual heterozygous for increased long arm length. Ascertainment was first made by length measurement. Increased length in one A1 chromosome is correlated with increased heterochromatin. Polymorphism is heritable. Heterochromatin is visualized by first denaturing the chromosomal DNA *in situ*, then staining with Giemsa. Details of this method are presented elsewhere¹²⁻¹⁴.

A family possessing an unusually long arm of chromosome A1 has been recently studied using these methods¹²⁻¹⁴. The results show that the extra length of the chromosome A1 long arm is caused by an increase (approximately $\times 2$) of centromeric associated heterochromatin (Fig. 2) which provides preliminary support for the arguments presented here and suggests that other minor variants may be causally related to polymorphisms restricted to heterochromatic areas. Another finding in support of this viewpoint is the high frequency of length variation associated with the A1, C9, E16 and Y chromosomes. These chromosomes all contain very large blocks of heterochromatin¹²⁻¹⁴. We are re-examining our families possessing these polymorphisms. If variations in length generally prove to be due to variation in the length of heterochromatin areas, this would explain the benign effect of most such variants on phenotype and hence their high frequency. Lastly, because the polymorphic homologue can now be readily identified by a simple staining technique, it should be a simple matter to determine its pattern of inheritance in kindreds. This should provide extremely useful material for the purpose of establishing gene linkage to specific chromosomes.

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Role for Ferredoxins in the Origin of Life and Biological Evolution

THE ferredoxins are members of a class of metalloproteins known as iron-sulphur proteins which act as electron carriers in such diverse biochemical processes as carbon metabolism, nitrogen fixation, photosynthesis and steroid hydroxylation^{1,2}. They are relatively small proteins with molecular weights of 6,000 to 12,000; they contain 2-8 atoms of iron and equivalent amounts of inorganic sulphur per molecule, transferring electrons at low redox potentials (-300 to -500 mV at pH 7) and having a characteristic electron paramagnetic resonance signal at $g=1.94$ in the reduced state. Ferredoxins have been found in a wide range of organisms, from the primitive anaerobic bacteria to all higher plants and animals. A knowledge of the changes in their amino-acid sequence³ is therefore of great value to the study of the evolution of these organisms.

We propose that a ferredoxin may have been among the earliest proteins formed and that ferredoxins may be very suitable for use in the study of the evolution from anaerobic to aerobic organisms. The ferredoxins from anaerobic bacteria have a very simple composition which seems to have developed into the more complex ferredoxins of the photosynthetic bacteria. The final stage of development was reached with the appearance of the primitive blue-green algae whose ferredoxin seems to be similar to those of all other algae and higher plants.

When life started, the principal mode of metabolism is thought to have been anaerobic fermentation of organic compounds which had formed abiogenically in the primordial environment^{4,5}. The ferredoxins probably played a significant role in the development of the fermentative bacteria. In present day organisms of this type such as the clostridia, the ferredoxins are simple, small proteins of fifty-five amino-acids and molecular weight 6,000 with a primitive polypeptide structure⁶. The amino-acid sequences of three of them are known⁷⁻⁹. These contain fourteen different types of amino-acids but only nine are common to all three ferredoxins. Significantly, the six amino-acids detected^{11,12} in the Murchison meteorite (which fell in Australia in 1969)¹⁰—glycine, alanine, valine, proline, glutamic acid and aspartic acid—constitute 64% of the total amino-acid content of *C. butyricum*⁸, while the nine "common" amino-acids (the six plus serine, cysteine

Unlike other electron-transferring proteins, such as cytochromes and flavoproteins, which have complex organic molecules in their active site, the ferredoxins have only iron and inorganic sulphur. Once the polypeptide chain of ferredoxin was formed, these could readily be added as an iron salt and sulphide, both of which were abundant in the primitive Earth. This process is known to occur readily in anaerobic conditions¹³.

In the evolution of green algae and higher plants from the blue-green algae, the oxygen-evolving photosynthetic system and the type 4 ferredoxins seem to have remained essentially unchanged. This observation supports the suggestion that plant and algal chloroplasts are the descendants of blue-green algae which lived symbiotically inside the early plant cells¹⁶. Thus all chloroplast ferredoxins would be derived from a common ancestor, namely blue-green algae.

(A)	Ala-	Thr-	Tyr-	Lys-	Val-	Thr-	Leu-	Lys-	Thr-	Pro-	Ser-	Gly-	Asp-	Gln-	Thr-	Ile-	Glu-	Cys-	Pro-	Asp-	20
(B)	Ala-	Ala-	Tyr-	Lys-	Val-	Thr-	Leu-	Val-	Thr-	Pro-	Thr-	Gly-	Asn-	Val-	Glu-	Phe-	Gln-	Cys-	Pro-	Asp-	20
(C)	Ala-	Ala-	Phe-	Lys-	Val-	Lys-	Leu-	Leu-	Thr-	Pro-	Asp-	Gly-	Pro-	Lys-	Glu-	Phe-	Gln-	Cys-	Pro-	Asp-	20
(D)	Ala-	Thr-	Tyr-	<u>Lys-</u>	<u>Val-</u>	<u>Lys-</u>	<u>Leu-</u>	<u>Val-</u>	<u>Thr-</u>	<u>Pro-</u>	<u>Ser-</u>	<u>Gly-</u>	<u>Gln-</u>	<u>Gln-</u>	<u>Glu-</u>	<u>Phe-</u>	<u>Gln-</u>	<u>Cys-</u>	<u>Pro-</u>	<u>Asp-</u>	20
(A)	Asp-	Thr-	Tyr-	Ile-	Leu-	Asp-	Ala-	Ala-	Glu-	Glu-	Ala-	Gly-	Leu-	Asp-	Leu-	Pro-	Tyr-	Ser-	Cys-	Arg-	40
(B)	Asp-	Val-	Tyr-	Ile-	Leu-	Asp-	Ala-	Ala-	Glu-	Glu-	Glu-	Gly-	Ile-	Asp-	Leu-	Pro-	Tyr-	Ser-	Cys-	Arg-	40
(C)	Asp-	Val-	Tyr-	Ile-	Leu-	Asp-	Gln-	Ala-	Glu-	Glu-	Leu-	Gly-	Ile-	Asp-	Leu-	Pro-	Tyr-	Ser-	Cys-	Arg-	40
(D)	Asp-	Val-	<u>Tyr-</u>	<u>Ile-</u>	<u>Leu-</u>	<u>Asp-</u>	<u>Gln-</u>	<u>Ala-</u>	<u>Glu-</u>	<u>Glu-</u>	<u>Val-</u>	<u>Gly-</u>	<u>Ile-</u>	<u>Asp-</u>	<u>Leu-</u>	<u>Pro-</u>	<u>Tyr-</u>	<u>Ser-</u>	<u>Cys-</u>	<u>Arg-</u>	40
(A)	Ala-	Gly-	Ala-	Cys-	Ser-	Ser-	Cys-	Ala-	Gly-	Lys-	Val-	Glu-	Ala-	Gly-	Thr-	Val-	Asp-	Gln-	Ser-	Asp-	60
(B)	Ala-	Gly-	Ser-	Cys-	Ser-	Ser-	Cys-	Ala-	Gly-	Lys-	Leu-	Lys-	Thr-	Gly-	Ser-	Leu-	Asn-	Gln-	Asp-	Asp-	60
(C)	Ala-	Gly-	Ser-	Cys-	Ser-	Ser-	Cys-	Ala-	Gly-	Lys-	Leu-	Val-	Glu-	Gly-	Asp-	Leu-	Asp-	Gln-	Ser-	Asp-	60
(D)	<u>Ala-</u>	<u>Gly-</u>	<u>Ser-</u>	<u>Cys-</u>	<u>Ser-</u>	<u>Ser-</u>	<u>Cys-</u>	<u>Ala-</u>	<u>Gly-</u>	<u>Lys-</u>	<u>Val-</u>	<u>Lys-</u>	<u>Val-</u>	<u>Gly-</u>	<u>Asp-</u>	<u>Val-</u>	<u>Asp-</u>	<u>Gln-</u>	<u>Ser-</u>	<u>Asp-</u>	60
(A)	Gln-	Ser-	Phe-	Leu-	Asp-	Asp-	Ser-	Gln-	Met-	Asp-	Gly-	Gly-	Phe-	Val-	Leu-	Thr-	Cys-	Val-	Ala-	Tyr-	80
(B)	Gln-	Ser-	Phe-	Leu-	Asp-	Asp-	Ser-	Gln-	Ile-	Asp-	Glu-	Gly-	Trp-	Val-	Leu-	Thr-	Cys-	Ala-	Ala-	Tyr-	80
(C)	Gln-	Ser-	Phe-	Leu-	Asp-	Asp-	Glu-	Gln-	Ile-	Glu-	Glu-	Gly-	Trp-	Val-	Leu-	Thr-	Cys-	Ala-	Ala-	Tyr-	80
(D)	Gly-	<u>Ser-</u>	<u>Phe-</u>	<u>Leu-</u>	<u>Asp-</u>	<u>Asp-</u>	<u>Glu-</u>	<u>Gln-</u>	<u>Ile-</u>	<u>Gly-</u>	<u>Glu-</u>	<u>Gly-</u>	<u>Trp-</u>	<u>Val-</u>	<u>Leu-</u>	<u>Thr-</u>	<u>Cys-</u>	<u>Val-</u>	<u>Ala-</u>	<u>Tyr-</u>	80
(A)	Pro-	Thr-	Ser-	Asp-	Cys-	Thr-	Ile-	Ala-	Thr-	His-	Lys-	Glu-	Glu-	Asp-	Leu-	Phe-					96
(B)	Pro-	Val-	Ser-	Asp-	Val-	Thr-	Ile-	Glu-	Thr-	His-	Lys-	Glu-	Glu-	Glu-	Leu-	Thr-	Ala				97
(C)	Pro-	Arg-	Ser-	Asp-	Val-	Val-	Ile-	Glu-	Thr-	His-	Lys-	Glu-	Glu-	Glu-	Leu-	Thr-	Ala				97
(D)	Pro-	Val-	Ser-	Asp-	Gly-	Thr-	Ile-	Glu-	Thr-	His-	Lys-	Glu-	Glu-	Glu-	<u>Leu-</u>	<u>Thr-</u>	<u>Ala</u>				97

Fig. 1 Sequences of type 4 (plant) ferredoxins. The underlined amino-acids, of which there are sixty, are invariant. A, *Scenedesmus*; B, spinach; C, *Leucaena*; D, taro.

The type 4 plant ferredoxins have molecular weights of

A proposed model¹⁹ for the active site of type 4 ferredoxins is shown in Fig. 2. Whether a similar type of unit is applicable to the other types of ferredoxin is unknown but it may be possible to extend the model to the 2-electron transfer ferredoxins of types 1, 2 and 3 as has been suggested by Gibson *et al.*²⁰

The occurrence of iron-sulphur proteins in animals has not been dealt with here because little is known about them at present. But because of the recent recognition of their ubiquitous occurrence and role in important reactions such as oxidative phosphorylation and hormone synthesis, they will undoubtedly be investigated intensively in the next few years. As an example, the hydroxylase iron-sulphur protein adrenodoxin from adrenal glands contains two iron atoms and two sulphur atoms and transfers one electron; its chromophore

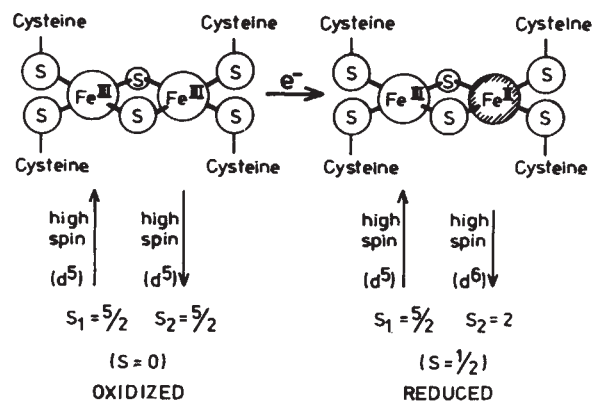


Fig. 2 Model of the iron-sulphur group in plant ferredoxins.

group seems very similar to the plant-type ferredoxins²¹ but its amino-acid sequence²² is quite dissimilar. Iron-sulphur proteins have also been detected in aerobic bacteria, fungi and yeasts¹.

Table 1 Amino-acid Composition of Type 4 (Plant) Ferredoxins

Amino-acid	1	2	3	4	5	6	7	8
Lysine	3	5	4	4	4	5	5	4
Histidine	2	1	1	1	2	1	1	1
Arginine	2	1	1	1	1	1	2	1
Tryptophan	1	0	0	0	0	1	1	1
Aspartic acid	11	14	12	9	14	10	10	13
Threonine	6	6	10	7	6	6	4	8
Serine	7	7	8	8	8	8	7	7
Glutamic acid	11	15	10	16	9	16	16	13
Proline	4	3	4	4	5	4	4	4
Glycine	6	6	7	9	9	8	6	6
Alanine	8	11	10	6	7	6	7	9
Cysteine	5	4	6	4	5	5	5	5
Valine	5	6	5	6	5	10	6	7
Methionine	1	1	1	1	2	0	0	0
Isoleucine	6	5	3	5	6	4	4	4
Leucine	9	9	7	8	7	6	9	8
Tyrosine	4	4	4	2	3	4	3	4
Phenylamine	2	3	3	4	4	2	3	2
Total amino-acids	93	101	96	95	97	97	96	97
References	*	23	24	18	25	26	27	28

1, *Microcystis* (blue-green alga); 2, *Bumilleriopsis* (yellow-green alga); 3, *Scenedesmus* (green alga); 4, *Equisetum* (horsetail; primitive plant); 5, fern (*Polystichum munitum*); 6, taro (*Colocasia*) (monocotyledonous higher plant); 7, *Leucaena glauca* (leguminous tree); 8, spinach (*Spinacea oleracea*) (dicotyledonous higher plant).

* K. T. Yasunobu, R. V. Smith, M. C. W. Evans and K. K. Rao, unpublished observations.

In conclusion, ferredoxins may have been among the simplest proteins, being first formed as a short polypeptide chain of twenty-six amino-acids comprising only nine different amino-acids which are easily formed in simulated primaevial conditions. It might have been possible for the primitive organism to utilize the amino-acids from its environment to synthesize this simple protein. Inorganic iron and sulphur were available in abundance and could readily combine with the apoprotein in a non-enzymatic reaction to form the chromophore group of a ferredoxin which could transfer electrons at reducing potentials of -420 mV similar to those of H_2 gas. From studies of amino-acid sequence the ferredoxins seem to have evolved from the anaerobic fermentative bacteria to the anaerobic photosynthetic bacteria and thence to oxygen evolving, photosynthetic organisms. At some stage, proteins similar to the ferredoxins were incorporated into the metabolic pathways of animals, yeasts and fungi, also catalysing

electron transfer reactions. Thus the ferredoxins are a class of proteins in which patterns of evolution can be followed.

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Structure of α -Keratin

Low angle X-ray diffraction is a powerful means of investigating long range axial order in fibrous protein structures, and α -keratin was one of the earliest materials to be studied by this method¹. The observed meridional and near-meridional reflexions have been indexed²⁻⁵ as orders of an axial period of $198 \text{ \AA}$, but there are indications^{6,7} that this might not be correct. This possibility has been investigated using a high resolution focusing X-ray camera⁸ to obtain diffraction

patterns from several native keratins and from certain heavy-atom derivatives of porcupine quill.

It is difficult to calibrate fibre patterns with sufficient precision to combine results from different specimens and for the purpose of comparison it was assumed that the well resolved meridional reflexion about 24.8 Å corresponded to the eighth order of a 198 Å axial period. Thus the reciprocal space coordinate ζ of this reflexion was taken as 0.0404 Å^{-1} in each case and the indices of the remaining reflexions calculated on this basis. The results (Table 1) show that the measurements on keratins from different sources and on different derivatives agree in general to within the estimated experimental error of $\pm 0.2 \times 10^{-3} \text{ Å}^{-1}$. The ζ -values calculated on the basis of a 198 Å axial period are also listed and it will be seen that there are discrepancies considerably in excess of experimental error between the observed and calculated values. It seems therefore that the axial period of α -keratin is not 198 Å, as is generally supposed.

From a consideration of the results given in Table 1 an alternative axial period of 470 Å was derived and it will be seen (Table 1) that this enables all the observed meridional reflexions to be indexed in a satisfactory manner. In no case does the difference between the observed ζ -value and that calculated on the basis of a 470 Å axial period exceed the estimated experimental error. Further measurements on non-meridional low angle reflexions and on near-meridional high angle reflexions indicate that an axial period of 470 Å is likely to provide a satisfactory basis for indexing the complete diffraction pattern. For comparison, the diffraction patterns of the materials listed in Table 1 were placed on a common scale but the absolute value of the axial period is known to increase with hydration². Measurements on dry porcupine quill dusted with a powdered calibrating substance give an absolute value of $470 \pm 3 \text{ Å}$ for the axial period in this material.

At low angles the prominent meridional reflexions fall on layer lines with indices (based on a 470 Å period) which satisfy the selection rule $l = 19m$, $19m \pm 5$ and $19m \pm 7$, where $m = 0, 1$ and 2 . This type of distribution is characteristic of a periodically distorted structure^{9,10}. A likely cause of such a distortion is that the fibrous elements (microfibrils) have an initial screw axis with 19 units in t turns of pitch $470/t \text{ Å}$ which is disturbed when close packing of the microfibrils takes place during biogenesis. Strong near-meridional diffraction occurs on $l = 17$ and the lateral coordinate of the maximum is such that it could only be due to a first order Bessel function. From the Bessel function selection rule for a helical structure with u units in t turns ($l = um + tn$; where m and n are integers)¹¹ it follows that with $l = 17$ and $u = 19$, $t = 2$ for $n = -1$. This helix is illustrated in Fig. 1.

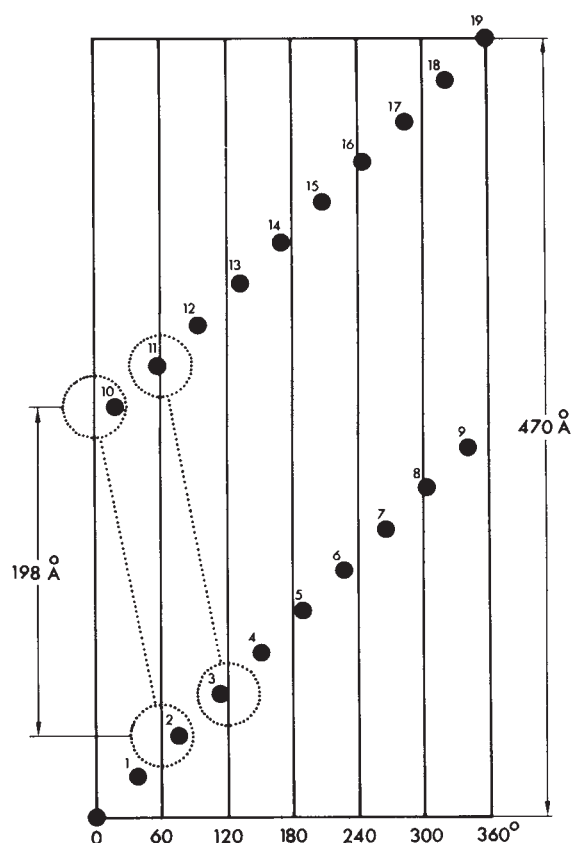


Fig. 1 Radial projection of the axial repeat of structure in the α -keratin microfibril. An isolated microfibril contains 19 units in 2 turns of a helix of pitch 235 Å giving an axial repeat of 470 Å. The vertical lines represent lines of contact with neighbouring microfibrils in a hexagonally close packed structure. Units separated by an axial translation of 198 Å (dotted lines) have almost identical environments and presumably similar distortions. The screw-sense of the helix cannot be determined directly from the X-ray data, but considerations of the way in which left handed coiled coil ropes could be packed side by side with a displacement of 24.8 Å suggest that better packing would be obtained with a right handed screw axis for the microfibril.

The origin of the 198 Å pseudo-period in the axial projection of the structure is immediately apparent from Fig. 1 where the contacts between a microfibril and its six nearest neighbours are indicated by vertical lines. Units separated by an axial translation of 198 Å have almost identical environments and

Table 1 ζ -Values of Meridional Reflexions in the X-ray Diffraction Pattern of α -Keratin (10^{-3} Å^{-1})

African porcupine quill									
Dry		14.8	25.4			40.4	55.3	80.9	95.7
Hydrated	10.8	14.9	25.5			40.4	55.1	80.5	95.2
Silver derivative	10.7	15.0	21.3		29.9	40.4		80.7	
Osmium derivative	10.8	14.7	25.4	29.8	34.1	40.4		80.8	95.7
Chromium derivative		14.9	25.6	29.7		40.4	55.1		
S. American porcupine quill	10.7	14.9	25.6	29.9		40.4	55.3	80.7	95.4
Lincoln wool fibres		15.0	25.6	29.8		40.4			
Mean ζ -value	10.8	14.9	21.3	25.5	29.8	34.1	40.4	55.2	80.7
Index with 198 Å period	2	3	4	5	6	7	8	11	16
Calculated ζ -value	10.1	15.2	20.2	25.3	30.3	35.4	40.4	55.6	80.8
Error	0.7	-0.3	1.1	0.2	-0.5	-1.3	0	-0.4	-0.1
Index with 470 Å period	5	7	10	12	14	16	19	26	38
Calculated ζ -value	10.6	14.9	21.3	25.5	29.8	34.0	40.4	55.3	80.8
Error	0.2	0	0	0	0	0.1	0	-0.1	-0.1

presumably therefore undergo similar distortions. The pseudo-period of 198 Å is thus a consequence of the pattern of distortion rather than an indication of molecular dimensions. The redistribution of intensity which occurs at low angles when α -keratin is hydrated⁷ therefore reflects a change in the nature of the distortion pattern at the surface of the microfibril.

Our conclusions have several implications for further studies of the structure of α -keratin. (a) The microfibril has a nineteen-fold rotation axis in projection and a radius ~ 36 Å¹², and so it is unlikely that the individual structural units could be resolved when viewed in axial projection in the electron microscope^{13,14}. (b) The interactions along the lines of contact between microfibrils are quite precise, suggesting that bridges exist between them or that the intervening matrix has, at least in part, a regular structure. (c) The microfibril in α -keratin is believed to be composed of molecules of molecular weight $\sim 45,000$ (ref. 15). If the number of such molecules associated with each unit in Fig. 1 were 1, 2 or 3 the corresponding microfibril radii would be, respectively, ~ 27 , 38 or 47 Å assuming a density of 1.3 g cm^{-3} . The value derived for two molecules per unit gives the best agreement with the estimate of 36 Å for the radius of the microfibril in African porcupine quill derived from the low angle equatorial X-ray pattern¹². A consideration of the distribution of intensity in the meridional pattern in various heavy atom derivatives, which will be discussed in detail elsewhere, suggests that the two molecules may be related by a horizontal dyad. (d) The prominent 5.16 Å arc in the high angle diffraction pattern does not index satisfactorily on a 198 Å axial period^{16,17} but seems to fall on the ninety-first layer line of the 470 Å period. Weaker arcs at 5.06 and 4.96 Å fall on the ninety-third and ninety-fifth layer lines respectively. The diffraction in this area originates from the coiled coil ropes in the interior of the microfibril¹² rather than from the surface and the screw axis is less likely to be disturbed by inter-microfibrillar interactions. The appearance of low order Bessel functions on $l=91, 93$ and 95 is to be expected from the selection rule $l=19m+2n$ for a helix with 19 units in 2 turns.

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Geometry of the Myosin Dimer

MONOMERIC myosin in aqueous solutions of high ionic strength is in rapid reversible equilibrium with a dimer species¹⁻³. Because this dimer is likely to be the initiating and possibly the building unit in the formation of the thick filament of muscle, its three dimensional geometry is of considerable importance.

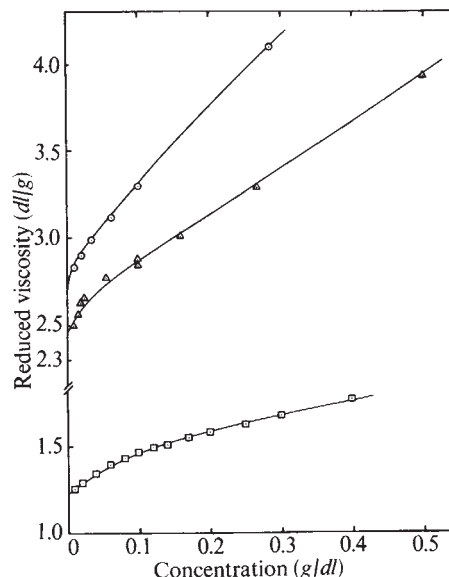


Fig. 1 The reduced viscosity as a function of concentration for myosin (Δ), LMM ($\square$) and rod ($\circ$) at 5.0°C . A 10 min tryptic digestion of myosin was used to isolate LMM, while insoluble cellulose-papain was used for limited digestion of myosin to yield rod^{13,14}. Both fragments were purified from low molecular weight components by fractionation on a column of 'Sephadex G-200'. All protein solutions were made up gravimetrically using solvents which had been passed through 'Millipore' filters. An Ostwald-type viscometer was used with an average shear gradient 300 s^{-1} . The outflow time for the solvent at 5.0°C was 146 s and the kinetic energy correction was found to be negligible. The solid curves represent the best computed fit to the experimental data (see text) with the corresponding parameters given in Table 1.

The solubility properties of light meromyosin (LMM) and "headless" myosin (rod), as well as X-ray⁴ and electron microscopic evidence⁵⁻⁸, suggest that the interaction sites responsible for the association of myosin reside primarily along the surface of its rod segment. Since the tendency for self-association of the myosin molecule is not completely repressed in high ionic strength media², both LMM and rod may also exist under these conditions in a rapid, reversible monomer $\rightleftharpoons$ dimer equilibrium. This equilibrium may have been overlooked since both fragments, like myosin, exhibit single peaks in sedimentation velocity experiments, thus satisfying the criteria for either an apparently monodisperse non-interacting system or a rapid, reversible monomer $\rightleftharpoons$ dimer equilibrium^{9,10}. In the case of myosin, the presence of a rapid, reversible monomer $\rightleftharpoons$ dimer association¹ was established only after careful sedimentation equilibrium study and computer analysis^{2,11,12}.

We have found that both LMM and rod self-associate in high ionic strength media by a rapid, reversible equilibrium of the monomer $\rightleftharpoons$ dimer type exhibited by myosin. This suggests that measurements of the asymmetry of these particles in dimerizing conditions could resolve the problem of whether the myosin dimer is formed through parallel or antiparallel association. The myosin molecule can be represented as a rod with a ball at one end. If two such structures are placed side by side in either parallel (head to head) or antiparallel (head to tail) arrangement and one of the two molecules is displaced

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axially with respect to its neighbour, for each displacement there are, for both LMM and rod, unique dimer structures which characterize the geometry of the parent myosin dimer. We have investigated the concentration dependence of the viscosity of LMM and rod in the ionic medium in which myosin is in a rapidly reversible monomer⇌dimer equilibrium.

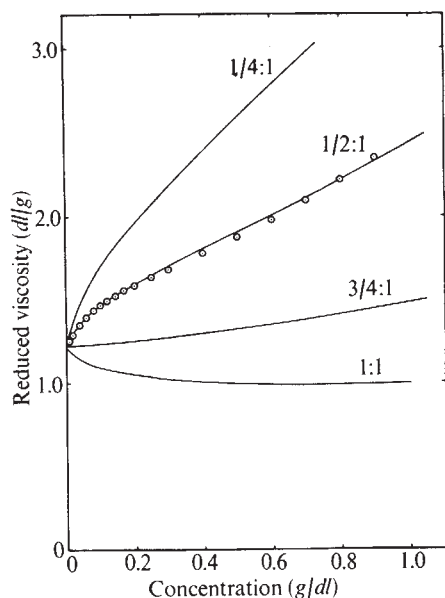


Fig. 2 The viscosity versus concentration dependence of light meromyosin (○) at 5.0° C. The solid curves represent the theoretical reduced viscosity versus concentration plots for LMM in a monomer ⇌ dimer equilibrium with respect to the specified dimer geometry. The parameters used to construct the theoretical plots are given in Tables 1 and 2. A value of 0.45 was assigned to the Huggins constant k' .

Plots of viscosity versus concentration of LMM, rod and myosin in 0.5 M KCl, 0.2 M PO₄ and 0.01 M EDTA, pH 7.3 (Fig. 1), all curve downwards at low concentrations in the manner expected for an associating system in which the associated species is more asymmetric than the monomer. In earlier studies^{13,14}, downward curvature was not detected since measurements were generally made at concentrations greater than 0.5 mg/ml. Moreover, there was no reason to assume that a monomer⇌dimer association reaction was occurring in these early studies. The downward curvatures of Fig. 1 cannot be due to binding of the protein to the capillary wall, since plots of the difference in outflow time between solution and solvent ($t-t_0$) versus protein concentration for these three particles extrapolated through zero at $c=0$ ¹⁵. The theoretical reduced viscosity versus concentration plots expected for a monomer⇌dimer equilibrium as a function of dimer geometry were calculated for light meromyosin (Fig. 2) by using the parameters given in Tables 1 and 2. The intrinsic viscosities of the dimer were evaluated from hydrodynamic studies of macromodels as described later. Assuming that a monomer⇌dimer equilibrium does exist and that the specific viscosity (η_{sp}) of the mixture will be an additive function of the specific viscosities of each of the species present, the specific viscosity of the equilibrium system can be expressed by the equation

$$\eta_{sp\text{ mixture}} = \sum_{i=1}^2 \eta_{sp_i}$$

where

$$\eta_{sp_i} = \left[[\eta_i] + k' [\eta_i]^2 c_i \right] c_i$$

c_i is the concentration of the i^{th} species (dl/g) and k' is the dimensionless Huggins constant which, for non-associating systems, has values between 0.4 and 0.5.

The experimental data in Fig. 1 have been fitted using various values of K (the equilibrium constant) and $[\eta]_d$ with k' equal to 0.45. The intrinsic viscosity of monomer, $[\eta]_m$, was obtained by extrapolation of the experimentally obtained reduced viscosities to infinite dilution. The derived values of $[\eta]_d$ and K which best fit the experimental points are given in Table 1 and the curves generated from them are shown in Fig. 1. In the case of myosin the value of 10 dl/g was assigned to the equilibrium constant, this being the value found by high speed equilibrium sedimentation².

Table 1 Hydrodynamic Parameters derived from Reduced Viscosity versus Concentration Dependence of Myosin and its Proteolytic Fragments LMM and Rod

Particle	$K(\text{dl/g})$	Theoretical (model studies) $[\eta]_m(\text{dl/g})$	Experimental $[\eta]_m(\text{dl/g})$	$[\eta]_d(\text{dl/g})$
LMM	5.52	0.910	1.23	1.90
Rod	27.5	1.73	2.65	3.42
Myosin	10.0 ⁽²⁾	—	2.45	3.11

To find the geometry of the dimer structure which would be consistent with the derived $[\eta]_d$ values of the three particles, we investigated the hydrodynamic behaviour of macromodels of differing dimer geometry¹⁵⁻¹⁸. The scaled up models were constructed from precision ground stainless steel rods of 0.317 cm diameter or from brass rods of 0.236 cm diameter. It was assumed that the LMM particle was 860 Å long and 20 Å in diameter and that the rod was 1290 Å long and 20 Å in diameter. Very close agreement was found between the experimental torque and that predicted by the Broersma expression for single rods of varying axial ratio¹⁸.

The axial ratio of an effective ellipsoid having the same volume as the dimer was calculated from measurements of the torque required to rotate the specific dimer model with unit angular velocity in a solution of invert sugar of viscosity 200 poises at 22° C. The intrinsic viscosities expected for the various dimer geometries were derived from the axial ratio utilizing the Perrin¹⁹ and Simha²⁰ equations and assuming apparent specific volumes of 0.710 and 0.728 for LMM (rod) and myosin, respectively¹. The intrinsic viscosities expected for the monomeric LMM and rod particles, based on modelling studies, were smaller than those observed experimentally (Table 1). This discrepancy probably arose from the assumption of an incorrect geometry of the monomer used in the construction of our models. We have normalized the viscosities obtained from the model studies to take into account the geometry differences (Table 2).

Table 2 Intrinsic Viscosities derived from Hydrodynamic Studies of Macromodels of Differing Dimer Geometry

LMM			Rod		
Fraction of overlap	$[\eta]_d(\text{dl/g})$	Dimer length (Å)	Fraction of overlap	$[\eta]_d(\text{dl/g})$	Dimer length (Å)
1.0	0.613	860	1.0	1.52	1290
0.75	1.23	1080	0.75	2.65	1610
0.50	1.82	1290	0.66	3.10	1720
0.25	2.74	1500	0.33	5.41	2150

In the case of LMM, the $[\eta]_d$ derived from the experimental curve is best fitted by a dimer geometry corresponding approximately to a 430 Å overlap. Similarly, in the case of the rod, the best dimer geometry corresponds approximately to a 780 Å overlap. Although various geometries for the myosin dimer would be consistent with the experimentally deduced dimer viscosities of LMM and rod taken separately, only one structure is consistent with the combined results from both particles. This structure is a parallel myosin dimer in which the head of one myosin molecule is displaced 430–510 Å with

respect to that of its neighbour. The intrinsic viscosities of myosin should be somewhat lower than those of the corresponding rod structures because of the presence of the globular head. The value of $[\eta]_d$ for myosin (Table 1) is therefore consistent with the parallel-type geometry.

These results do not preclude the possibility that other dimeric structures exist, but in the conditions of these experiments the most likely structure is the dimer described. Moreover, Herbert and Carlson have shown, from their light scattering results, that the z-average radius of gyration of myosin has a concentration dependence that strongly supports the view that this parallel dimer is formed on self-association (unpublished results).

The geometry of the myosin dimer deduced here has interesting implications in the assembly of the thick filament of muscle. It is clear from the model of the thick filament proposed by Pepe⁶ that the structure can be constructed in all its features entirely from this specific parallel-type dimer in which the head of one molecule is displaced about 430 Å with respect to its neighbour. The bare central region of the filament would be constructed by antiparallel (tail to tail) association of the dimer units. The filament would grow by head to tail addition of the dimers. Moreover, such a dimer has been invoked by one of us as the basic transducing element of skeletal muscle²¹. This proposal requires that the head of one molecule is in juxtaposition with the hinge section (trypsin sensitive region) of its neighbour.

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Extended Sleep and Performance

MOST studies of the psychological consequences of sleep have been concerned with the way in which sleep deprivation decreases performance^{1,2}. Little has been done to discover the relationship between extended sleep and performance. It

may therefore be of interest to describe a study in which unnaturally extended sleep has been found to be followed by decreased performance in certain tasks.

Twelve normal male college students, who regularly slept 7–8 h nightly, were requested to abstain from drugs including alcohol for at least 5 days before their first night in the experiment and until after the last. They typically averaged 7–8 h of sleep per night according to their sleep logs. On the days of the experiment latency from the last meal was controlled and subjects were instructed not to nap or to drink caffeinated beverages after 1800 h and to maintain their usual level of physical activity. Subjects were informed that the purpose of the experiment was to investigate the relation between sleep and personality.

An adaptation night preceded 2 control nights of habitual sleep and 2 nights of extended sleep with a 1 day recovery period between the last two conditions. Subjects slept in pairs and were always awakened at 0900 h following both conditions, but were not told this, and were served 6 ounces of fruit juice as a partial nutritional control for blood sugar level. In the extended sleep condition subjects retired at 2200 h and in the control condition they were in bed by 2200 h engaged in quiet activities until 0100 h when they were requested to sleep.

Calculation and vigilance tasks similar to those used by Wilkinson *et al.*³ and a complex motor performance (pinball, Gottlieb "swing along") task were administered as performance measures at a fixed time to control for circadian effects. Order of presentation for the pinball and vigilance tasks as well as conditions of sleep were counterbalanced across subjects. The 50 min calculation task which involved adding 100 columns of five two-digit numbers was given 30 min after awakening. The number of sums added incorrectly and the time required to complete it was scored. Following the calculation task a 45 min pinball task and a 45 min version of the Wilkinson vigilance task² were administered simultaneously and alternately to each of the pair of subjects. For the vigilance task the measure of performance taken was the combined number of signals missed (omissions) and incorrectly detected (false reports). For the pinball task average score per game was scored, efficient performance being represented by a high score. Electroencephalographic, electromyographic and electro-oculographic activity was recorded and the records scored according to standard procedures⁴.

Table 1 shows the number of minutes spent in each sleep stage, total sleep and wakefulness time and total time spent in bed during both nights of each sleep condition. Because of technical difficulties, the adaptation night was substituted for one of the control nights in computing the various means in Table 1. On nights of extended sleep, the mean time allowed for sleep was 10.6 h and, of this, subjects slept 9.1 h on average, with a range of 7.5–10.7 h. Most of the time spent in bed awake on these nights was generally attributable to long sleep latencies which averaged 47 min, a smaller portion of this period being due to terminal wakefulness, which averaged 21 min. On control nights the mean time allowed for sleep was 7.6 h and, of this, subjects slept 7.1 h on average with a range of 6.2–7.8 h. Wakefulness on these nights was minimal for most subjects. Comparisons between the two conditions for the amount of time spent in each sleep stage were reported previously⁵.

Decrements in performance with extended sleep were significant for two measures: combined number of omissions and false reports on the vigilance task (Table 2; $P < 0.005$, one-tailed Wilcoxon test) and average score per game on the pinball task (Table 2; $P < 0.025$, one-tailed Wilcoxon test). There were no significant differences in performance between the control and extended sleep condition on the calculation task for either errors or speed of calculation (control condition: mean errors = 5.7, s.d. 3.53, mean speed = 25.4 min, s.d. 6.4; extended sleep condition: mean errors = 6.3, s.d. 2.03, mean speed = 24.2 min, s.d. 3.5).

It seems unlikely that the effect of prolonged sleep was due to variations in nutritional intake or less activity consequent to

Table 1 Sleep Latency, Wakefulness, Minutes Spent in Each Sleep Stage, Total Sleep Time and Total Time in Bed for Control (C) and Extended Sleep (E) Conditions

Subject	Sleep latency (min)		Wakefulness during sleep (min)		Terminal wakefulness (min)		Stage 1		Stage 2		Stage 3-4		Stage REM		Total sleep time (h)	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
1	2	35	17	3	0	3	48	54	201	252	85	86	100	169	7.2	9.4
2	10	11	0	6	80	7	24	26	173	254	79	118	93	192	6.2	9.9
3	3	110	21	8	12	67	19	30	190	262	67	68	112	88	6.5	7.5
4	0	107	18	21	0	0	30	32	197	212	72	103	110	161	6.8	8.5
5	7	45	1	5	0	19	31	25	217	296	86	103	111	140	7.7	9.4
6	0	32	37	8	22	68	38	24	205	271	95	104	76	138	6.8	8.8
7	22	27	18	86	0	13	45	65	254	251	61	104	57	87	7.0	8.4
8	2	14	46	16	0	18	14	34	155	297	145	82	99	166	6.8	9.7
9	2	2	3	10	0	0	22	33	247	288	97	124	103	194	7.8	10.7
10	3	89	9	7	0	0	26	21	216	290	95	83	126	161	7.7	9.3
11	2	54	13	31	8	17	34	53	191	227	122	121	94	135	7.3	8.9
12	2	36	4	74	0	4	24	70	185	293	141	81	107	75	7.6	8.7
Means	6	47	16	22	10	18	30	39	203	266	95	98	99	142	7.1	9.1

a prolonged period in bed, for there were controls for these factors, but it is possible that the effect is related to sleep onset occurring at an earlier phase of the circadian rhythm. Because wakefulness tended to increase in the extended sleep condition, this might account for the decrements in performance. This increase, however, was primarily in sleep latency at the beginning of the night. Increased wakefulness during sleep increased at a rate of only 0.2 min/h. The increase in terminal wakefulness of 8 min does not seem to be a crucial factor, especially because testing on pinball and vigilance tasks did not begin until 75 min after subjects rose from bed. As in sleep deprivation experiments⁶ and a previous study on extended sleep², accuracy on the subject-paced calculation task was insensitive to changes in amount of sleep, whereas the experimenter-paced pinball and vigilance tasks proved to be sensitive measures of impaired performance following manipulation of sleep variables.

Table 2 Average Performance on the Vigilance and Pinball Tasks after Two Nights of the Control (C) and Extended Sleep (E) Conditions

Subject	Combined errors of commission and omission on the vigilance task		Average score per game on the pinball task	
	C	E	C	E
1	7.5	8	592	643.5
2	9	8.5	557.5	583.5
3	14	15	464	516.5
4	4	1.5	559.5	550
5	11.5	21	571	515.5
6	10.5	15.5	654.5	516
7	3	9	638	378
8	25.5	38	525	469
9	2.5	10	533	481
10	10.5	10.5	531.5	522.5
11	9.5	16	586	505.5
12	20.5	41	548	490.5
S.D.	6.8	12	51.2	63.7
Means	10.6	16	563.5	514

It is well known that sleep deprivation impairs waking behaviour⁷. This investigation indicates that deviating from a habitual regime of sleep by extending sleep is detrimental to subsequent waking behaviour as well. We suggest that any

general theory of sleep should attempt to explain both phenomena.

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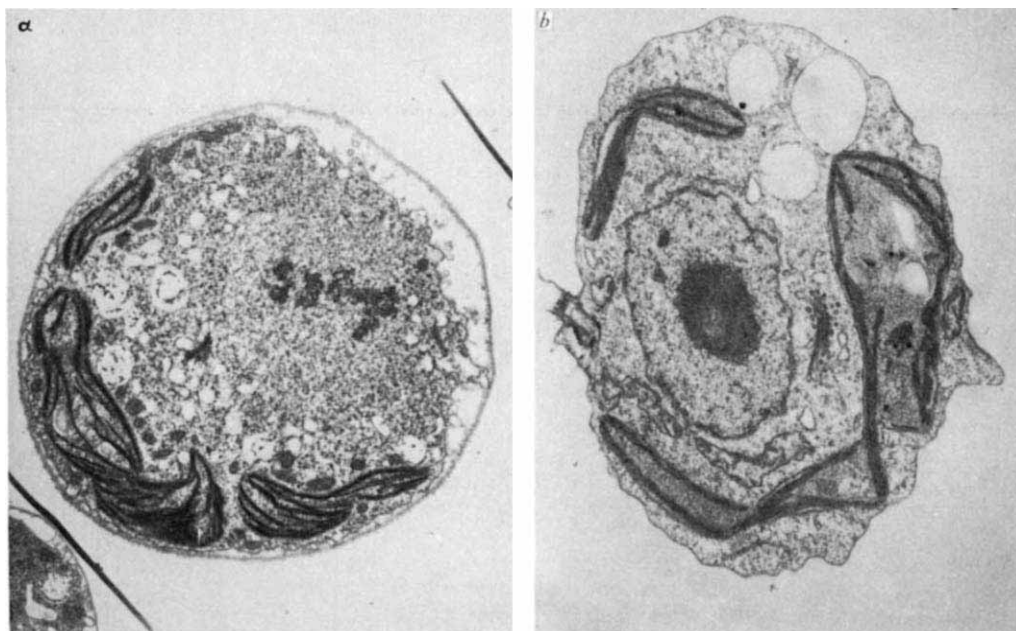
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Single Cell Protein and the Exploitation of Mutant Algae lacking Cell Walls

MICROBIAL cells are potentially attractive sources of protein food, but this usefulness is at present limited, not merely by technical and economic difficulties¹⁻⁴, but also because the indigestibility of the cell walls limits the extent to which they

Fig. 1 Longitudinal section of (a) a wild type cell, (b) a mutant CW15, lacking a cell wall. Note cell is bound only by a plasma membrane. (Electron micrograph $\times 7,200$.)



can be incorporated directly into human food. Indeed it has been suggested that microbial cells⁵ and the alga *Chlorella*⁶ cannot be used in human food without removal of the cell walls. For algae, there are two ways of overcoming this difficulty—either the material can be fed to ruminants, or mechanical, chemical or enzymatic techniques⁷ can be used for the fractionation and removal of the cell walls. Enzymatic methods are as yet inefficient and, whereas industrial scale production in Czechoslovakia involves mechanical disruption of the algal cells with Ballotini beads, Japanese commercial production involves chemical extraction. Although all these methods are easy and efficient on a laboratory scale, on an industrial scale problems of fractionation are as formidable as those of cultivation². Furthermore, the cost involved militates against a more general utilization of this food source. There is now evidence that by genetic manipulation it is possible in some instances to overcome these problems posed by the presence of the cell wall.

Table 1 Amino-acid Composition of Wild Type Cells and of a Mutant Strain CW15 Lacking Cell Walls

Amino-acid	mg in WT	mg in CW15
Lysine	0.78	0.85
Histidine	0.21	0.29
Arginine	0.81	0.86
Aspartic acid	1.11	1.21
Threonine	0.60	0.64
Serine	0.54	0.53
Glutamic acid	1.30	1.27
Proline	0.60	0.57
Glycine	0.68	0.64
Alanine	0.92	0.90
Half cystine	0.09	0.07
Valine	0.76	0.73
Methionine	0.35	0.49
Isoleucine	0.61	0.48
Leucine	1.08	1.08
Tyrosine	0.50	0.55
Phenylalanine	0.67	0.63
Total protein	11.61 mg	11.79 mg

20 mg of acetone-ether washed cells was used in each instance.

Following treatment with the chemical mutagen N-methyl-N'-nitro-N-nitrosoguanidine we have isolated in the unicellular green alga *Chlamydomonas reinhardtii* a large number of mutants with various defects in cell wall biogenesis (ref. 8 and

unpublished work). These are readily distinguished from wild type cells by their altered colony morphology. A few of these mutants produce no detectable quantities of cell wall (Fig. 1); in spite of this, some can show comparable growth rates to the wild type and in all respects they seem to be perfectly viable and normal when grown in liquid culture. Furthermore the cells retain ample mechanical strength to allow aeration, stirring, and harvesting by centrifugal separation. Since the nature and quantity of protein are of primary relevance in the context of single cell protein production comparative assays of protein content and of amino-acid composition of one of the mutant strains and of the wild type were undertaken. The results are given in Table 1. The percentage of protein in the wall-less mutant is comparable with that in the wild type although the wall itself does contain protein. The total weight of the wall, however, is small in comparison with that of the whole cell. The differences in amino-acid composition between the two strains are of little significance.

The potential value of such mutant forms of green algae lacking cell walls is immediately apparent in that it overcomes one of the most serious problems facing those who have sought to exploit them as a source of single cell protein⁹. While the particular mutants of *C. reinhardtii* may have no significance as sources of single cell protein there is clearly every incentive for undertaking programmes to induce and isolate comparable mutant strains, not only of the algae now being grown on a commercial scale⁴ but also of other microorganisms.

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BOOK REVIEWS

Success Stories

Contemporary British Chemists. By W. A. Campbell and N. N. Greenwood. Pp. 286. (Taylor and Francis: London, June 1971.) £5.

IN a technological, numerate, post-Dainton society there is great advantage in producing graduates with some understanding of a physical science such as chemistry, without expecting them to rely on it too heavily in their post-university careers. To ignore details of the second law of thermodynamics matters little, provided there is some notion of the thought processes used in deriving it. Chemistry has become a core subject or, according to one eminent chemist in industry, has ceased to exist.

This point of view represents a revolution against the consensus of the past. Until the late fifties of this century, professors of chemistry walked ten feet high. The aspiring young chemist, thankful for intelligence and luck which allowed him to be accepted for and surviving an honours course, emerged at the end bursting with facts, more or less well digested, but certain that society will reward him appropriately for the years spent in dark satanic labs. These beautiful simplicities eroded under the twin pressures of facts and industrial forces. As the number of facts multiplied, it became simply impossible to digest more than a fraction in the three to four years available. A substantial portion of industry began its clamour for more and more of new style scientists, whose education would render them more suitable for managerial positions.

The response of chemical educators has been the notion of chemistry not as a vocational but as an educational discipline. Improvements in teaching methods both at school and at university resulted in enormously improved curricula and methodologies, and the new views have obtained if not universal at least widespread approval. Yet a paradoxical situation remains. To make science as attractive as possible to schoolchildren and undergraduates, their enthusiasm has to be aroused, but not to such a pitch that they should elect, in the vast majority of cases, to carry on with science after graduation. For the individual, education in science represents no longer a vocation for the discipline, but rather one for worldly success. Irrespective of any other consideration, the functional success of such an argument requires that for those who study chemistry, or the

sciences generally, the resulting qualification should open all doors from Whitehall to Throgmorton Street and offer a level of comfort the young aspirant would like to be accustomed to.

The main argument of this book is that it is indeed possible to scale professional heights with a chemistry degree, even in fields that have little to do with chemistry itself. Its method is to present scientific and personal biographies of 145 successful people—all but five of them men—based on 180 questionnaires sent out by the authors. While the aim is excellent and the presentation of the book first class, the very complexities of changing society have introduced a number of difficulties. The selection of top people, not even within a profession but based loosely on it, is always a hazardous procedure: since under our system it is extremely unlikely for anybody to become obviously eminent very early in life, the success stories are products of the very educational ideas that have lost ground. Thus the vast majority of the 145 are professors of chemistry: undeniably important figures in the scientific landscape but hardly calculated to convey the message of a wide variety of career choices. The successful chemist appears to be doing what chemists have always been doing: research. *Se monumentum requiris, circumspice*. Yet this year the highest level of graduate unemployment since the war will certainly force all new science graduates to explore careers outside the confines of their discipline.

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Astrochemistry

Geochemical Exploration of the Moon and Planets. By I. Adler and J. I. Trombka. Vol. 3. Pp. x+243. (Springer-Verlag: Berlin and New York. 1970.) 58 DM; \$16.

THIS book represents an attempt to review past and present efforts to determine the geochemical composition of the Moon and planets. It is essentially concerned with the techniques of geochemical exploration and, as such, could have been given a more specific and informative title. That it contains a fairly extensive review in its 239 pages cannot be denied; however, the organization of this review leaves much to be desired.

The book is arranged, or more accurately, is arbitrarily divided, into six chapters. The first chapter is an introduction which briefly summarizes some of the scientific problems to be solved by exploration of the Moon and

planets. The next chapter is entitled "Instruments Used for Compositional Exploration", and describes the instrumentation and experimental results of the Surveyor, Ranger and early Luna spacecraft. The third chapter, "Instruments and Techniques under Development", contains much of the meat of the book in terms of the range of techniques discussed. The authors describe experimental arrangements developed, and being developed, to carry out chemical analysis of the planets using X-ray spectroscopy, X-ray diffraction, neutron activation and mass spectrometry in surface experiments, and X-ray, γ -ray, α -particle and infrared spectroscopy in orbital experiments. The fourth chapter, "Apollo Surface Missions and the Lunar Receiving Laboratory Program", is concerned with the analysis of the returned lunar samples. Presumably because of the breadth of the overall programme, the discussion is restricted to describing NASA's Lunar Receiving Laboratory programme. This is done in unnecessary detail and makes very tedious reading. For example (when describing the Gas Analysis Laboratory), "This laboratory is located on a mezzanine above the Vacuum Laboratory on the third floor of the LRL". The whole chapter, including a description of the preliminary results of Apollo 11, seems to have been taken, with minimal editing or reorganization by the authors, from NASA reports. The fifth chapter, "Data Processing and Analysis", discusses in some detail the problems of data acquisition and analysis from remote environments. The final chapter, "Orbital and Surface Exploration Systems after Early Apollo", discusses a number of projected integrated missions incorporating and extending the techniques discussed earlier.

It is inevitable that much of the book will be rapidly outdated by events in the fast changing field of space exploration. Unfortunately, the way the book is organized accentuates this problem, and in reading it I found myself constantly thinking of how the arrangement of topics could have been improved to make something of more lasting value. The authors do not seem to have set out with a well defined purpose for the book, and this is probably its major flaw. As an honest-to-goodness reference manual of current techniques for space exploration, it is too diffuse. The discussions of scientific results are also scattered about the book and of necessity too cursory to be of any value. As a historical record it suffers from the fact that much of the

record is of projected experiments.

People seeking an introduction to the techniques of remote geochemical exploration may find this book useful. They will not find that it has the polish of a good textbook. Perhaps that is too much to expect of an up-to-date review.

GRENVILLE TURNER

Search for Spectra

The Spectra and Structure of Simple Free Radicals. By Gerhard Herzberg. Pp. xi+226. (Cornell University: Ithaca and London, June 1971.) £5.25.

THE 1968 Baker lectures at Cornell University by Gerhard Herzberg, now published in this slim volume, fulfil the ambition of the author to write an introduction to molecular spectroscopy. Few would argue that he is the greatest exponent of the subject still in active research, and on the basis of his three comprehensive books one might suppose that anything he does not know about molecular spectroscopy is not worth knowing.

The book has a title and a sub-title: an introduction to spectroscopy. The latter gives a better representation of its character because free radicals are used only to illustrate general features of molecular spectroscopy and little emphasis is placed on any unique features. In fact the definition of radical which has been adopted ("any species with a short life in the gas phase under normal laboratory conditions") would not be generally accepted in other branches of chemistry. For example, the molecule C_2 is included but not NO. The omission of detailed discussion of electron spin resonance makes the title slightly inappropriate.

The introductory chapter covers some of the special problems that arise in the measurement of the spectra of transient species, although the book as a whole has a theoretical rather than an experimental approach to the subject. There is an enjoyable account of the search for the spectrum of CH_2 which was stimulated by the observation in 1941 of some unidentified lines in the spectrum of a comet. These were finally assigned to molecule C_3 but CH_2 was eventually tracked down in 1961 in the flash photolysis of diazomethane.

The main part of the book is divided into three sections: diatomics, linear polyatomics and non-linear polyatomics. As an indication of the level of the book, the diatomic chapter gives an account of rotation-electronic coupling, the symmetry properties of rotational levels, of Hund's coupling cases *a* and *b* (but not the less important cases) and the rotational structure of electronic bands. It can therefore be seen that the book goes much further than is usual in undergraduate spectroscopy texts.

The chapters on polyatomic radicals give very good accounts of vibronic interactions (the Renner and Jahn-Teller effects) and of vibration-rotation interactions (Λ doubling and Coriolis interaction). There is also an extensive discussion of molecular orbital correlation diagrams and their relevance to the understanding of state symmetries and molecular shapes.

Finally the book is completed by a short chapter on radiationless processes: dissociation, predissociation and recombination. A description of the mechanism of these processes is given and a discussion of selection rules, but no reference has been made to the recent work on predissociation line widths which has been undertaken in several different laboratories.

The book has been elegantly produced and is a pleasure to read. It is a pity that it is so highly priced, as it should be widely used as a textbook for graduate courses in spectroscopy.

J. N. MURRELL

Chemical Mutagenesis

Chemical Mutagenesis in Mammals and Man. Edited by F. Vogel and G. Röhrborn. Pp. xiv+519. (Springer-Verlag: Berlin and New York, 1970.) 124 DM; \$34.10.

VARIOUS expert committees have recently emphasized the critical need for mutagenicity testing of chemicals before their registration for commercial or other uses. These committees include the Task Force on Research Planning in Environmental Health Science, March, 1970; the Food and Drug Administration Advisory Committee for Protocols on Safety Evaluation, Panel on Reproduction Studies in the Safety Evaluation of Food Additives and Pesticide Residues, December, 1969; and the Advisory Panel on the Mutagenicity of Pesticides, the Secretary's Commission on Pesticides and their Relationship to Environmental Health, December, 1969. Such recommendations not only reflect critical concerns as to the public health implications of chemical mutagenesis, but also the recent development of a variety of test methods, data from some of which have a high degree of presumptive human relevance.

Chemical Mutagenesis in Mammals and Man is the first of several recent books addressed to this problem with particular emphasis on the importance of *in vivo* mammalian systems in testing for chemical mutagens. This book is a compilation of papers presented at a symposium held on October 7, 1969, in Mainz, Germany. The editors, Drs Vogel and Röhrborn, are admirably qualified for this purpose, as they are internationally recognized and respected workers in the respective fields of

population genetics and mutagenicity testing in mammals.

The book contains thirty chapters by authors who include many well known authorities in chemical mutagenesis, including Barthelmess, Ehling, Kuhlmann, Legator, Malling, and Chu. Most chapters deal comprehensively with the specifics of mutagenicity testing in mammalian systems. The editors have also contributed chapters on genetic monitoring of human populations and on the dominant lethal assay in rodents, and also a very useful summary. The stylistic heterogeneity of the book, which the editors have wisely preserved, makes for dynamic and stimulating reading.

The editors are to be warmly congratulated for their timeliness in publishing this volume, which will be of great value, not only to research workers in the field, but also to the chemical and pharmaceutical industries, and to government agencies.

SAMUEL S. EPSTEIN

Designing Libraries

New Library Design: Guide Lines to Planning Academic Library Buildings. By Stephen Langmead and Margaret Beckman. Pp. ix+117. (Wiley: Toronto and Chichester, August 1971.) £5.75.

THE ideas for this book came from the authors' collaboration as project architect and systems librarian for the McLaughlin new library building at the University of Guelph, Canada. They have subsequently acted as consultants to several other Canadian libraries and they lecture in library architecture at the Graduate School of Library and Information Science at the University of Western Ontario. Experience taught them that there are certain steps fundamental to the planning process which are ignored at peril and others which, while not absolutely essential, greatly smooth the passage of a plan through its various stages. In consequence they have set down a systematic schedule of steps to be taken from beginning to end of the planning of a large university library. The resulting guidelines enable anyone planning a new academic library to identify the problems which will need to be solved, make it quite clear who will need to be consulted and why, and provide many of the factual details and most up-to-date accepted standards which both architect and librarian will need. As an illustrative example the McLaughlin Library and its planning brief are described, with added diagrams and photographs.

This must surely be a classic case of joint consultation, the very antithesis of the Columbia Law Library which was described as "reflecting, by its symmetry and balance, the triumph of the Architect over the Librarian". Here the librarian takes his proper place as a

qualified person who is well able to organize and administer the extremely complex functions of a modern library intent on communication as well as conservation, who understands the needs of faculty and students, and who controls a management system which designs and evaluates his services. The architect is a professionally competent adviser, planner, designer, coordinator, cost estimator, arbitrator and supervisor. The two clearly respect each other's contribution. The library design favoured is a completely flexible modular plan, flexibility being carried to the point of specially designed handling equipment which can move a fully loaded bookstack.

There is nothing essentially new about library design in the book. The novelty exists in putting it all down on paper so concisely and usefully to produce a book which will take an immediate place as a standard text.

W. ASHWORTH

Processing Pictures

Picture Language Machines. Edited by S. Kanef. (Proceedings of a Conference held at the Australian National University, Canberra, February 24-28, 1969.) Pp. xiv+425. (Academic: London and New York, December 1970.) £4.50.

ALTHOUGH the title of this book reflects an overall concern for some general understanding of what would characterize an acceptable picture processing system, individual papers span a variety of topics. The collection starts with four reviews, by Narasimhan on picture languages, Bobrow on question-answer systems, Clowes on a layman's guide to Chomsky, and Jacks on design principles for an industrial graphics system. Then jackets are taken off and the fun begins. The general effect of the book is that of a broadside. Away with the restrictive attitudes of classical pattern recognition with its naive hope for classification based on independent feature extraction. In with the view that any picture processing system worthy of its salt must come to grips with the full range of deep problems associated with systems which can bring to bear an open ended corpus of knowledge about depicted domains to the interpretation of what is depicted. It is this last theme, aspects of which are referred to as "an ability to appropriately articulate structure" (paraphrasing Clowes) and an "ability to use models which are isomorphic to those of the human interacting with the system" (paraphrasing Stanton) which is emphasized in various forms in different papers. It is this theme which motivates the inclusion of the particular review papers and also interesting

papers on topics such as natural language behaviour, the role of learning in picture processing, and on dynamic data structuring.

Several stimulating discussions have the theme of the desirability of problems posed by picture processing systems which would make use of descriptions to generate appropriate and different algorithmic behaviour in different contexts.

I found the collection heavy going, largely because of the prolix style of many of the authors—why use a phrase when a paragraph will do? By contrast, the unedited discussions at the end of each paper have an Alice in Wonderland flavour, but like Alice they are sometimes remarkably rewarding.

Nevertheless, given that one is not reading to obtain answers but rather to seek for sensible questions, and those not just about picture processing, the book is well worth the effort involved in reading much of it two or three times.

E. W. ELCOCK

Flying Mammals

Biology of Bats, Vol. 1. Edited by William A. Wimsatt. Pp. xii+406. (Academic: New York and London, December 1970.) £11.65.

Biology of Bats, Vol. 2. Edited by William A. Wimsatt. Pp. xv+477. (Academic: New York and London, January 1971.) £12.15; \$26.

EVERY biologist secretly believes that his "group" is underrated by the scientific community which fails to appreciate the variety of its forms, the beauty of their adaptations and the importance of the problems posed. Those who study bats are of course quite different: they know their subjects get a raw deal. One has only to analyse any general zoology text or university course: bats fly and thus have unique skeletal modifications (differing from those of pterodactyls and birds) and most use echolocation to catch insects (the rest eat fruit). But whoever quotes a desert bat in an essay on water regulation?

The reasons for all this are quite simple. In addition to the irrational popular repugnance or indifference due to lack of confrontation, there is a natural reluctance to tackle the large and diffuse literature. The lack of a general account of bat biology has been remarkable. Allen's excellent monograph of 1938 was republished, unaltered twenty-four years later and still seems to be in demand after a third of a century. Yet its treatment is semi-popular and necessarily superficial; more recent texts, in German and French, are even shorter.

The deficiency is now being made good by a treatise, edited by William

Wimsatt, of which two volumes are now published. It would be unfair to try to assess the scope of the work at present—a third volume and possibly a fourth are planned but no information is yet available on their probable contents. One can only hope at this stage that current omissions will be made good when the work is complete. The present two volumes contain seventeen essays by twelve authors, all American and all authoritative. Each volume carries an introduction by the editor, who has not yet contributed to the text, and detailed author and subject indices. They are beautifully produced, with copious illustrations of high quality, except for some old engravings which have not reproduced well, and one diminutive pair of X-ray photographs.

The first volume has ten chapters on a wide variety of topics and they are necessarily very variable in length. The first and longest chapter, on bat origins and evolution by Jepsen, deserves special mention. It contains a more detailed description of *Icaronycteris index* (the oldest and most complete fossil bat) than has hitherto been published and is illustrated by numerous superb stereo photographs. For many workers this material alone will justify the purchase of this volume. In addition the author gives a general discussion of the Chiroptera that forms an excellent introduction to the whole treatise. The general taxonomy of the order has not changed since Miller (1907), but Jepsen adds some stimulating ideas on relationships. He also argues that flying and gliding make different demands on the body so that bats could never have been gliders.

The second chapter is on karyotypic trends by Baker, and then follow three chapters by Vaughan, giving detailed descriptions of the skeletal and muscular systems respectively, and a discussion of flight patterns and aerodynamics. This last is surprisingly brief although new work is known to be in hand. The remaining five chapters are diverse. Orr writes on development, prenatal and postnatal, giving only two and a half pages to prenatal aspects, which will presumably be expanded later under reproduction. Griffin reviews migration and homing, Davis discusses hibernation ecology and physiological ecology and Lyman deals with thermoregulation and metabolism, but not the metabolic and thermal problems of active flight. Finally Rosenbaum gives a detailed comparative account of the urinary system.

The second volume, although longer, contains only seven chapters, all but the last being closely related. Quay, writing on the integument and its derivatives, mentions, but does not describe, the elaborate and varied facial structures. Sensory aspects of the skin lead natur-

ally into the central nervous system, discussed by Henson, the peripheral nervous system (Quay), the ear and audition (Henson), vision, olfaction and taste (Suthers) and finally to a brief account of the pineal by Quay. Other endocrine aspects will presumably follow later. The volume concludes with an enormous consideration (131 pages) of bats in relation to human health. While no one, least of all those who work with living bats, should doubt the importance of this subject, its place in this volume and the mystery about future plans suggest poor coherence in the whole work.

But for the rest we must (and will eagerly) wait and see. I have high hopes and whatever the outcome one thing seems certain: that the current increase in work on bats, which has occurred in spite of the lack of a book of this sort, will gain much impetus now that it has appeared. DAVID PYE

Character and Attitude

Character Structure and Impulsiveness. By David Kipnis. (Personality and Psychopathology: a Series of Monographs, Texts and Treatises.) Pp. xi+133. (Academic: New York and London, March 1971.) \$7.95; £3.75.

Attitude and Attitude Change. By Harry C. Triandis. (Wiley Foundations of Social Psychology Series.) Pp. xiii+232. (Wiley: New York and London, April 1971.) £3.50 boards; £1.65 paper.

THE first of these books reports a series of interrelated studies, all based on the author's scale of impulsiveness, a scale which correlates both with Gough's socialization scale and the extraversion scale of the MPI. The scale concentrates on masculine outdoor hobbies, and all studies are therefore wisely limited to male subjects. Kipnis starts with the interesting and important idea that diagnostic instruments in psychology should be used like diagnostic tests in medicine, that is not only to describe the current state of a person but to show how to improve the score of those with a deficit on the test.

After a series of studies designed to establish the validity of his instrument he implements this action-orientation in the following way: high scorers on impulsiveness are underachievers in college examinations; they are also highly motivated by material incentives. Kipnis, accordingly established an ingenious field experiment with proper controls, in which impulsive underachievers were paid money for doing their mathematics course. The results show that the more intelligent impulsive students improved their grade in this condition, which had, however, no effect on the less intelligent. Although the role of intelligence as a

moderator of other personality predispositions is interesting, it receives no theoretical treatment in this book, which is altogether deficient on theory. Furthermore, the effect disappeared when payment stopped. The intended "cure" may, for all one knows, only have strengthened the original "illness". Here, as elsewhere, nothing would have been more practical than a good theory to deal with such doubts.

The study of attitudes continues to dominate social psychology and social psychological publications. At least a dozen books on attitude and attitude change have been published during the past year. Triandis's contribution—*Attitude and Attitude Change*—is in the textbook category. As one would expect from this author, it is workmanlike, clearly written and thoroughly up-to-date. The text is refreshingly catholic in approach and goes beyond the currently most fashionable debate about consistency theories. Instructors and students will find the student assignments at the end of each chapter particularly useful; they are imaginative guides to self-instruction and encourage students to think about the underlying assumptions in attitude research. There is a bibliography of well over five hundred items. MARIE JAHODA

Quantum Text

Practical Quantum Mechanics I. By Siegfried Flügge. (Die Grundlehren der Mathematischen Wissenschaften in Einzeldarstellungen, Band 177.) Pp. xiv+341. (Springer-Verlag: Berlin and New York, 1971.) 70 DM; \$20.30.

Practical Quantum Mechanics II. By Siegfried Flügge. (Die Grundlehren der Mathematischen Wissenschaften in Einzeldarstellungen, Band 178.) Pp. xii+287. (Springer-Verlag: Berlin and New York, 1971.) 60 DM; \$17.40.

ANYONE who has taught a course of quantum mechanics knows the difficulty of providing practical examples which are within the mathematical competence of the students and can be completed in a reasonable time. In these two volumes, which have been designed as a unified work, will be found 219 problems, together with their solutions, which will greatly extend the repertoire. They are all of moderate length and will be accessible to any student who can recognize the Bessel equation and consult references for properties of its solutions when these are required.

The first volume deals exclusively with one-body problems without spin. Many one dimensional and three dimensional potentials are introduced and the problems of calculating wavefunctions for bound states and for elastic scatter-

ing are well represented. Applications are made to some specific situations in atomic and nuclear physics, with numerical values given to all the constants so as to emphasize the order of magnitude of the effects. The problems illustrating scattering resonances and Regge poles are especially welcome. In the second volume the problems cover a wider range and include illustrations of the introduction of spin, the interactions between two and three particles, quantum statistics and the Dirac relativistic equation with shorter sections on non-stationary problems and radiation theory. It will be very useful to have problems on tensor forces, the Tietz approximation in Thomas-Fermi theory and on helicity. Between the two volumes most of the features of elementary quantum mechanics which have to be compared with the corresponding features of classical mechanics are introduced and discussed.

Faced with so many useful problems and elegant solutions I am reluctant to turn to the limitations of this collection. It has to be said, however, that the problems are all of textbook type and deal with the solution of the differential equations for the wavefunctions and the subsequent calculation of properties. The classical techniques of mathematical analysis are emphasized. Matrix techniques, which are often better illustrations of real problems and are extensively used in modern self-consistent field treatments of atoms and molecules, scarcely appear and there is no group theory. The diagram techniques of modern perturbation theories appear briefly in only one solution. As an introduction to the practical problems of quantum theory and to the most used mathematical methods this is a very lopsided and unrepresentative compilation. As a preparation for courses on specialized aspects of quantum theory probably the examples on nuclear theory will be considered most adequate. The student interested in atomic and molecular physics, in field theory or in solid state physics will find few examples that he cannot find elsewhere.

The volumes have been well produced with many well designed graphs. The English style has some reminders of the German of the earlier edition, but the book has been rewritten. The purist will object to the abbreviation of "having no complete set of eigenvectors" to "having no eigenvectors" in some matrix problems.

For the teacher who selects carefully and can supplement from other sources, this will be a valuable and much used addition to the armoury. For the unaided student who learns best by working out examples and attempting open-ended problems the practical introduction to quantum mechanics still has to be written. G. G. HALL

CORRESPONDENCE

Tolerable Pollution

SIR,—I have read with great interest Dr Samuel S. Epstein's article entitled "Control of Chemical Pollutants" (*Nature*, **228**, 816; 1970). Many of the points made by Dr Epstein are very well taken and need emphasis. Unfortunately, however, some of the concepts that he expresses need to be examined more closely and carefully thought through.

For instance, in the determination of "efficacy" the practising physician may have entirely different concepts than the academician who is not dealing with patients on a daily basis. To the academician there is probably no place for a placebo. To the harassed physician trying to bring solace to the whining, complaining patient the placebo may have a most important, though limited, role. I remember clearly a female patient I once had of 70+ years who demanded of me a "tonic". "Tonics," it should be remembered, were practically a part of the culture of people in this age range. Finding no "tonics" in the formulary of the hospital where I was working, I prescribed an innocuous "bladder mixture" made up of potassium iodide and hyoscyamus. Much to my surprise, the patient returned at her next visit lauding the "efficacy" of this "tonic" to the skies and insisted that it be renewed. Obviously this was not a material that I should be permitted to market as "Dr E's famous tonic", but was there any harm in its use in these circumstances? Who, then, is to determine efficacy? Should flavours, colours, antirancidity materials or other such materials be permitted in foods? Do they have "efficacy"? Some might well argue that foods should be eaten only with their natural inherent flavours and colours and that the addition of artificial (or even natural) flavours or colours

does not improve the food's efficacy. Rancid food should simply be thrown out. What about the present uproar over the use of tolbutamide in diabetics? Even cyclamates are thought by many physicians to have value in the treatment of obesity and diabetes. Who is to make these important determinations?

Turning to the concepts of carcinogenicity, teratogenicity, and mutagenicity, there are few people who do not believe that we should control them to the best of our ability. However, as I pointed out in my Ramazzini oration (*J. Occ. Med.*, **13**, 161; 1971), the concept of zero is a mathematical one. As our analytic techniques become more and more sensitive, it will ultimately be necessary for us to establish a "practical" zero. In essence this will mean that some finite, measurable quantity will be determined to be "tolerable". Thus a "tolerance" for carcinogens, teratogens, and mutagens will be established. Precedence for this approach has been established in the milk and water sanitation fields. Here a definite number of organisms are allowed, that is, are considered tolerable. It was recognized that to set the levels at zero organisms could well have eliminated these essentials from human consumption. Thus recognizing the impracticality of the so-called Delaney clause and devoting research efforts to a serious attempt to establish tolerances for carcinogens seems a more sensible approach than simply extending this concept to teratogens and mutagens. This approach has been taken by Truhaut and an international committee in attempting to establish a tolerance for aflatoxins in foods.

Lastly, the concept of "disinterested" scientists needs carefully to be examined. What, truly, do we mean by a "disinterested" scientist? Is he "disinterested" because he is an academician?

After all, Pasteur made what is probably one of the most important discoveries to mankind when he was working for the French wine industry. Is the scientist directly engaged in or dependent for support from a federal bureaucracy "disinterested" in its perpetuation? Can a scientist's views be modified as he lives longer and becomes more mature and knowledgeable? Is this change in viewpoint due to his having lost his disinterested status?

The concepts I expressed in my editorial on environmental carcinogenesis (*Cancer Res.*, **22**, 395; 1962) seem today to need re-emphasis. We do need to control significant exposures of the general population to carcinogens, teratogens, and mutagens. After all, today we do not have any real comprehension of what the teratogenic or mutagenic rates are in the human race as a whole. For all we know, these rates may be on the downswing now because of man's ability to overcome some of the vicissitudes of his former existence. A rereading of the "Report on the Sanitary Condition of the Labouring Population of Great Britain" by Edwin Chadwick, 1842 (edit. by M. W. Flinn, Edinburgh University Press, 1965) should reassure us on the real progress we have made in just a little over 100 years. We still, nonetheless, live in a real world, fraught with real and imminent dangers which we must do our best to minimize in a real and practical way.

Yours faithfully,

R. E. ECKARDT
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Obituary

Wendell Meredith Stanley

WENDELL MEREDITH STANLEY, Professor of Molecular Biology and of Biochemistry at the University of California, Berkeley, died suddenly, aged 66, on June 15 in Spain.

His career in biochemistry and molecular biology began at the University of Illinois in 1926 when, as a graduate student of the organic chemist, Roger Adams, he synthesized a series of fatty acids and correlated their structures and physical properties with effectiveness against the leprosy bacillus. After earning his PhD degree in 1929, Stanley

went to Germany as a National Research Council Fellow in the laboratory of Professor Heinrich Wieland, where he worked on sterols of yeast. Returning to the United States in 1931, he worked for a year at the Rockefeller Institute for Medical Research in New York with cell physiologist W. J. V. Osterhout, on model chemical systems that simulated cell membranes.

In 1932, Stanley moved to the Rockefeller Institute Laboratories in Princeton, New Jersey. There he began his studies on the chemistry of viruses which culminated in 1935 with the crystallization of tobacco mosaic virus

(TMV), the first of these disease agents to be obtained in such a form. Not content with the isolation and crystallization of TMV, he proceeded with a small group of colleagues to develop systematically and imaginatively an entirely new field of science: the biology, chemistry, and physics of viruses. From these efforts, and work elsewhere, the chemical composition and morphology of a variety of viruses were defined in molecular terms. Stanley's initial studies on the chemical modification of viruses later provided the foundation for inactivating viruses for vaccine production and for studies on molecular



genetics. With the onset of the Second World War, his group turned to animal viruses and their fundamental studies on

the purification and characterization of influenza virus led to the preparation of a safe and effective vaccine.

Stanley joined the University of California at Berkeley where, in 1948, he founded the Virus Laboratory and built a new Department of Biochemistry which he served as Chairman until 1953. Subsequently he initiated the formation of the Department of Virology in 1958 which he chaired until 1964 when it was expanded to become the present Department of Molecular Biology. In these academic departments, Stanley promoted and fostered the blending of teaching and research in a most fruitful way. His own interests were directed toward the conquest of viral diseases and the potentialities of viruses in the induction of cancer. He encouraged research in this area and exerted considerable influence in programmes on the national level. His contributions to cancer research were recognized by two

awards from the American Cancer Society, one in 1959 and another in 1963 and by his selection as President of the Tenth International Cancer Congress, Houston, Texas, in 1970.

Many honours came to Stanley over the years: elections to honorary societies, distinguished lectureships, about a score of honorary degrees, and numerous medals and prizes including the Nobel Prize in Chemistry in 1946. In his lifetime, he had three different and equally productive careers: first as a bold, imaginative scientist whose research revolutionized biological thought; second as an outstanding educational administrator who recruited, supported and inspired many young scientists; and third as a distinguished statesman who played a major role in shaping enlightened national and international policy with regard to scientific research on cancer and the role of viruses.

British Diary

Monday, September 13

Fundamentals of Software for Computer Engineers (vacation school, thirteen days) Institution of Electrical Engineers, in association with the Institution of Electronic and Radio Engineers, at the University of Manchester.

Surface Chemistry of Oxides (three-day Discussion) Faraday Society, in the Chemistry Department, Brunel University, Uxbridge, Middlesex.

Tuesday, September 14

5th Annual Solid State Devices Conference (three days) Institute of Physics and the Physical Society, in collaboration with the Institution of Electrical Engineers, the Institution of Electronic and Radio Engineers, the Institute of Electrical and Electronic Engineers, at the University of Lancaster.

Flexographic Printing Inks (7.30 p.m.) Oil and Colour Chemists' Association, at the Griffin Hotel, Boar Lane, Leeds.

Wednesday, September 15

Centrifugal Methods of Particle Size Analysis (2.30 p.m.) Society for Analytical Chemistry, North East Section and the Particle Size Analysis Group, at Newcastle University, Room L.101, Mertz Court, Queen Victoria Road, Newcastle-upon-Tyne.

Formulation of Two Epoxy Paints (4.30 p.m.) Mr A. McWilliam, Oil and Colour Chemists' Association, at the Manchester Literary and Philosophical Society, 36 George Street, Manchester 1. (Student Lecture.)

Thursday, September 16

Insect/Plant Relationships (two-day symposium) Royal Entomological Society of London, at the Imperial College of

Science Mechanical Engineering Lecture Theatre, Exhibition Road, London SW7.

Modern Analytical Techniques for Bio-Active Substances (two-day meeting) Society for Analytical Chemistry, at the University of Surrey, Guildford, Surrey.

Friday, September 17

The Work of the Government Chemist (7 p.m.) Dr H. Egan, Society for Analytical Chemistry, Western Region, in the Chemistry Department, University of Bristol.

Sunday, September 19

Electrical Measurement Practice (vacation school, thirteen days) Institution of Electrical Engineers, in association with the Institution of Electronic and Radio Engineers, at the University of York.

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